CONSIDERATION OF THE HAWAIIAN COLLECTOR URCHIN,

TRIPNEUSTES GRATILLA, AS A BIOCONTROL AGENT

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I AT MĀNOA IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTERS OF SCIENCE

IN

MARINE BIOLOGY

JULY 2018

BY

CHARLEY E. WESTBROOK

Thesis Committee:

Brian W. Bowen, Chairperson
Robert J. Toonen
Mary Hagedorn

Key Words: Invasive, Algae, Biocontrol, Feeding Preference

ACKNOWLEDGEMENTS

I express my gratitude to R. J. Toonen, B. W. Bowen, M. Hagedorn, R. R. Ringang, S. M. A. Cantero, as well as the field team from the state of Hawai'i's Division of Aquatic Resources from the Division of Land and Natural Resource (D. Cohen, J. Blodgett, B. Neilson, A. Purvus, F. Mancini, T. Walker, B. Stubbs, D. LeVault, C. Gewecke, V. Calabrese, K. Brittain, N. Rodriguez, S. Johnson) and The Nature Conservancy (R. Carr, H. Lynch, J. Dennis, K. Fujitani) who contributed to the completion of this study. Support for this project came in part from the National Science Foundation (NSF) Grant #OCE-0623678 and the National Oceanic and Atmospheric Administration's National Marine Sanctuaries Partnership MOA #2005-008/66882 to R. J. Toonen. R. R. Ringang and S. M. A. Cantero were supported as research interns under the Undergraduate Mentoring in Biological Sciences (URM) Grant (NSF #0829272). Additional support was provided by the Hawai'i Institute of Marine Biology's Lord Scholarship. I would also like to express my appreciation to members of the To Bo Lab at the Hawai'i Institute of Marine Biology, especially A. Dudoit, E. Johnston and G. Johnson for their encouragement along with R. Coleman, Z. Forsman and I. Knapp for their advice with data analysis. Ultimately, the bulk of my thanks go to my advisors and committee members B. W. Bowen, R. J. Toonen and M. Hagedorn for their support, tutelage and patience through my bourgeoning career as a researcher. Thank you all!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A_i:	Initial blotted-dry mass of algae
A_f:	Final blotted-dry mass of algae
AC_i:	Initial blotted-dry mass of control algae
AC_f:	Final blotted-dry mass of control algae
ANCOVA:	Analysis of Covariance
ANOVA:	Analysis of Variance
DAR:	Division of Aquatic Resources
HSD:	Honest Significant Difference
K. clade B.	*Kappaphycus clade B*
L:	Lagoon
min:	Minute
ns:	not significant
pers. comm.	Personal Communication
R:	Reef Flat
S:	Reef Slope
SAP:	Special Activities Permit
SE:	Standard Error
spp:	species pluralis
TD:	Test Diameter
TNC:	The Nature Conservancy
wk:	week
wt:	weight
yr:	year

CHAPTER 1. INTRODUCTION

Coral reefs and their significance

Coral reefs are a vital part of tropical marine ecosystems. Despite only making up about 0.5% of the ocean's surface area, coral reefs host approximately a quarter of marine species making them one of the most diverse ecosystems on Earth (McAllister, 1991; Spalding & Grenfell, 1997). They have been recognized for the myriad of goods and ecosystem services that they provide, which include: coastal protection via wave attenuation (Reguero et al. 2018), supplying recreational and subsistence fisheries (Hawkins & Roberts, 2004; Kuster et al. 2005), contributing to local economy through ecotourism (Asafu-Adjaye & Tapsuwan, 2008), as well as holding a vast diversity of compounds with pharmaceutical applications and medical potential (Erwin et al. 2010; Leal et al. 2013). Tens of millions of people rely on coral reef fisheries for food (McManus et al. 2000). Coral reefs have also contributed to the spiritual and cultural values of many countries, which are often reflected in the sustainable harvesting and responsible management of the natural resources in those areas (Doulman, 1993; Friedlander et al. 2000). However, for coral reefs to continue providing these goods and services, their habitat must be maintained in a human-dominated world (Moberg & Folke, 1999).

The necessity for herbivory

Grazers are an essential component to shallow-water coral reef communities. Not only do herbivorous organisms facilitate the flow of nutrients through the food web, they also contribute to increased biodiversity and primary productivity by removing algal species that would shade and otherwise outcompete other photosynthetic organisms (Carpenter, 1986). Without browsing organisms on coral reefs, macrophytic algae could begin to smother and displace coral colonies by limiting water flow, reducing light availability and by preventing new coral recruits from finding appropriate habitat to settle on (McCook et al. 2001; Burkepile & Hay, 2008; Murphy & Richmond, 2016). In the absence of sufficient grazing pressure, coral reefs may be unable to recover from the combination of natural and anthropogenic stressors (Hughes, 1994; McManus et al. 2000). Few cases highlight the essential role of the herbivorous functional group on coral reef ecosystems as notoriously as the critical depression in grazing pressure that occurred in the Caribbean during the 1980s. Overfishing in the Caribbean left the majority of the grazing burden on native urchins, *Diadema antillarum* (Hughes, 1994; Jackson et al. 2001). However, when a pathogen extirpated the Caribbean's urchin population, there were few functional grazers left in

the community to control algal growth (Lessios et al. 1988). Consequently, coral reefs in the Caribbean underwent a phase shift to algae dominated reefs, an ecological catastrophe that is still afflicting the Caribbean 30 years later (Lessios, 2016). The aforementioned case highlights the essential role of herbivores in maintaining a balanced reef community. A higher diversity and abundance of grazers increases a coral reef's resilience to invasive algae and is often synonymous with increased reef health (Elmqvist et al. 2003). Certain marine protected areas, with exceptionally large communities of grazing fish, are completely devoid of invasive species (Sheppard et al. 2012).

Impacts of invasive algae

Coral reef ecosystems are afflicted by multiple high profile anthropogenic stressors such as climate change (Wilkinson, 1999; Hoegh-Guldberg et al. 2007; Selkoe et al. 2008), pollution (Donahue et al. 2001; Hédouin et al. 2011) and resource exploitation (Munro, 1983; Jackson et al. 2001). A less recognized threat to the deterioration of coral reef communities is the establishment of invasive species, most notably algae. The primary origins of alien invasive algae have been identified as hull-fouling and aquaculture (Williams & Smith, 2007). Further compounding the risk of invasive algal blooms are the aggressive rates of propagation that certain macroalgae exhibit via fragmentation (Smith & Walters, 2002). Vegetative fragmentation accelerates the rates of spread of alien invasive algae, which have been reported to range anywhere between hundreds of meters to hundreds of kilometers per year from the point of introduction (Rodgers & Cox, 1999; Lyons & Scheibling, 2009). If alien algae become invasive on reefs they can compete with corals and inhibit their growth through a variety of mechanisms, which include overgrowth, shading, abrasion, chemical effects and substrate monopolization (McCook et al. 2001). Thick algal mats have even been observed to induce hypoxic environments below their canopy, smothering the corals trapped underneath (Murphy & Richmond, 2016). Invasive algae blooms further compromise the health of coral reefs by undermining the ecological processes that contribute to their resilience, thereby crippling their ability to recover from perturbation. The capacity of coral reefs to persist and regenerate can be jeopardized by the encumbrance of fleshy algae. If the invasive seaweeds outcompete coral spat for settlement space, larval colonization can fail thereby impeding reef replenishment by new coral recruits (Bellwood et al. 2004; Kuffner et al. 2006). If the algae's invasion continues without interference, reefs can experience a phase shift in which the benthos becomes dominated

by alien algae rather than native corals (Hughes, 1994). Exacerbating the issue of phase shifts are the behaviors of many herbivorous fishes that tend to avoid reefs overgrown by macrophytic algae (McClanahan et al. 1999; Smith et al. 2004). This loss of herbivores from a coral reef is likely to precipitate the expansion of an invasive algal bloom. If algal blooms persist on a reef for too long, corals will erode thereby deteriorating the three dimensional structure of the ecosystem; this degrades the reefs physical beauty, impacts community assemblages due to habitat loss and diminishes the reef's potential to function as a breakwater and protect nearby shoreline (Done, 1992). The majority of ecological studies performed on invasive algae have revealed that they cause multiple deleterious effects on coral reefs (Williams & Smith, 2007).

Additional research

In an effort to mitigate the impacts of invasive species, protect coral reefs and bolster their overall resilience, this study will investigate the use of biological control in management strategies. Debates have arisen over the efficacy of exotic biocontrol agents due to the inherent difficulty in predicting potential non-target species effects (Louda & Stiling, 2004; Cowie, 2010). Biocontrol strategies used in the agriculture sectors have been criticized for being reflexive and lacking sufficient evaluations of the cost and benefits of releasing new species in the ecosystem (Michaud, 2002). In some cases however, collateral damages to certain native species have been deemed an acceptable trade off of biological control if the benefits of removing an invasive species and its deleterious effects outweighs the cost to species of low conservation value (Hill, 1998; McConnachie et al. 2003; Atalah et al. 2013a). A survey of species released for biocontrol efforts in Puerto Rico since the early 1900's revealed that 20% were failures, nearly 12% were successful, 23% were partially successful and the remaining 45% were reported to have unknown success rates due to insufficient follow up studies (Cruz & Segarra, 1992). Although the use of biocontrol agents may appear enticing, it is important to study the candidate's life history and feeding behavior to justify its deployment. If the biocontrol's impact is incomplete, or non-specific to the target invasive species, then its use should be reconsidered (Wagner et al. 2009). Among the various qualities desired for biocontrol agents, one of the more contentious is their feeding behavior (Chang & Kareiva, 1999). Some argue that exotic biocontrol species should be specialists by acting antagonistically to the specific target only (Lafferty & Kuris, 1996; Cowie, 2010), while others have reported successes

using generalist biocontrol agents (Symondson et al. 2002; Stiling & Cornelissen, 2005). In the Caribbean, management strategies against crop pests have considered the use of biological control a successful alternative to pesticides and other toxic approaches of removing nuisance species (Cruz & Segarra, 1992). Approaches to water treatment in South Africa have considered studies of microbial biocontrol to demonstrate potential in suppressing harmful algal blooms and a desirable substitute to using toxic copper-based algicides (Gumbo et al. 2009). Occasionally, when manual removal is ineffective and chemical treatments are unavailable, biological control can become the only option for invasive species removal (McConnachie et al. 2003). A series of successfully implemented classical biocontrol efforts against insects and arachnids in agriculture have been reviewed (Caltagirone, 1981; Symondson et al. 2002). Although many studies have examined the application and potential development of biocontrol efforts in terrestrial systems, comparatively few investigations have been conducted for marine or estuarine systems (Lafferty & Kuris, 1996). Hoddle (2004) contends that the biological control remains a valuable yet understudied approach for dealing with exotic species invasions. Nevertheless, some notable studies have presented promising biocontrol agents to combat the spread of pests such as: native seastars against invasive seastars (Parry, 2017), native urchins against invasive kelp (Atalah et al. 2013a), native anemones, seasatars, sea urchins, hermit crabs and gastropods against biofouling organisms (Enright et al. 1984; Ross et al. 2004; Atalah et al. 2013b; Atalah et al. 2016), native urchins against nuisance gastropods (Malavé et al. 2012), native groupers against invasive lionfish (Mumby et al. 2011), sea slugs against invasive algae (Coquillard et al. 2000), exotic barnacles against invasive crab (Goddard et al. 2005), bacteria against algal bloom (Gumbo et al. 2008), crustaceans against disease vectoring snails (Hofkin et al. 1991).

This assessment will examine the collector urchin's, *Tripneustes gratilla*'s, feeding behavior and thus its potential to remove invasive algae in the Hawaiian Islands. Reports have suggested the use of a variety of urchin species for biocontrol efforts against a broad range of pests, such as: Kelp (Atalah et al. 2013a), fouling organisms (Lodeiros & García, 2004; Atalah et al. 2016), red algae (Conklin & Smith, 2005) and gastropods (Malavé et al. 2012). For the Hawaiian Islands however, further elucidation of the collector urchin's potential use as a biological control against alien macrophytic algae is warranted. This study is not an example of classical biocontrol, as the urchin is not exotic to the affected ecosystem. Rather, the strategy being investigated would lend itself to an augmentative biocontrol approach (Atalah et al. 2013a), because collector urchins are

indigenous to the Archipelago. Several other biocontrol reports have advocated for the use indigenous species to suppress pests as they do not introduce exotic organisms into the system and therefore have a lower risk of non-target effects (Lafferty & Kuris, 1996; Sigee et al. 1999; Ross et al. 2004; Gumbo et al. 2008; Atalah et al. 2013a; Atalah et al. 2015; Parry, 2017).

CHAPTER 2: SURVIVORSHIP AND FEEDING PREFERENCES AMONG SIZE CLASSES OF OUTPLANTED SEA URCHINS, *TRIPNEUSTES GRATILLA*, AND POSSIBLE USES AS BIOCONTROL FOR INVASIVE ALIEN ALGAE

This chapter may be cited as:

Westbrook C.E., R.R. Ringang, S.M.A. Cantero, HDAR & TTNC Urchin Team, R.J. Toonen. 2015. Survivorship and feeding preferences among size classes of outplanted sea urchins, *Tripneustes gratilla*, and possible use as biocontrol for invasive alien algae. PeerJ 3:e1235; DOI 10.7717/peerj.1235

Abstract

We investigate the survivorship, growth and diet preferences of hatchery-raised juvenile urchins, *Tripneustes gratilla*, to evaluate the efficacy of their use as biocontrol agents in the efforts to reduce alien invasive algae. In flow-through tanks, we measured urchin growth rates, feeding rates and feeding preferences among diets of the most common invasive algae found in Kāneʻohe Bay, Hawaiʻi: *Acanthophora spicifera, Gracilaria salicornia, Eucheuma denticulatum* and *Kappaphycus clade B*. Post-transport survivorship of outplanted urchins was measured in paired open and closed cages in three different reef environments (lagoon, reef flat and reef slope) for a month. Survivorship in closed cages was highest on the reef flat (~75%), and intermediate in the lagoon and reef slope (~50%). In contrast, open cages showed similar survivorship on the reef flat and in the lagoon, but only 20% of juvenile urchins survived in open cages placed on the reef slope. Urchins grew significantly faster on diets of *G. salicornia* (1.58 mm/week ± 0.14 SE) and *Kappaphycus clade B* (1.69 ± 0.14 mm/wk) than on *E. denticulatum* (0.97 ± 0.14 mm/wk), with intermediate growth when fed on *A. spicifera* (1.23 ± 0.11 mm/wk). Interestingly, urchins display size-specific feeding preferences. In non-choice feeding trials, small urchins (17.5–22.5 mm test diameter) consumed *G. salicornia* fastest (6.08 g/day ± 0.19 SE), with *A. spicifera* (4.25 ± 0.02 g/day) and *Kappaphycus clade B* (3.83 ± 0.02 g/day) intermediate, and *E. denticulatum* was clearly the least consumed (2.32 ± 0.37 g/day). Medium-sized (29.8–43.8 mm) urchins likewise preferentially consumed *G. salicornia* (12.60 ± 0.08 g/day), with less clear differences among the other species in which *E. denticulatum* was still consumed least (9.35 ± 0.90 g/day).

In contrast, large urchins (45.0–65.0 mm) showed no significant preferences among the different algae species at all (12.43–15.24 g/day). Overall consumption rates in non-choice trials were roughly equal to those in the choice trials, but differences among feeding rates on each species were not predictive of feeding preferences when urchins were presented all four species simultaneously. In the choice feeding trials, both small and medium urchins clearly preferred *A. spicifera* over all other algae (roughly twice as much consumed as any other species). Again, however, differences were less pronounced among adult urchins, with adults showing a significant preference for *A. spicifera* and *Kappaphycus clade B* compared to the other two algal species. These findings indicate that outplanted urchins are surviving on the reef flats and eating a variety of alien invasive algae as intended. Although juvenile urchins show stronger feeding preferences, these animals grow quickly, and adult urchins are more generalist herbivores that consume all four alien invasive algae.

Introduction

Within the last 70 years, Kāneʻohe Bay has become home to many introduced and invasive algal species, whose aggressive growth has smothered corals and overgrown many patch reefs across the bay (Coles et al. 2002; Conklin & Smith, 2005; Smith et al 2004, Stimson et al. 2001; Russel & Balazs, 2009; Bahr et al. 2015). Some of these alien algae species were introduced intentionally, whereas others have unknown origins (are cryptogenic), but appear to have gained a foothold in Kāneʻohe Bay thanks to a combination of reduced grazing intensity and high nutrient influx as a result of sewage discharge into the bay (Stimson et al. 2001). Among the most obvious and ecologically dominant of these invasive algal species are *Kappaphycus* clade B, *Eucheuma denticulatum, Gracilaria salicornia*, and *Acanthophora spicifera.* The species level taxonomy of *Kappaphycus* remains a subject of contention (Conklin et al. 2009; Sherwood et al. 2010). Due to the uncertain nomenclature of *Kappaphycus* in the literature, it was suggested we use the most contemporary denomination of the alga (despite the prospect of its name changing again, this is the best we could do at the time), henceforth it should and will be referred to as *Kappaphycus* clade B in this study (K. Conklin & A. Sherwood pers. comm.).

Native species of algae which once dominated the Bay (Stimson et al. 2001; Smith et al. 2004; Conklin & Smith 2005; Stimson et al. 2007) have become comparatively rare as the

rhodophytes *K.* clade B and *G. salicornia* both spread rapidly across Kāneʻohe Bay after their introduction, and are now found at high abundance throughout the Bay (Hunter & Evans 1995; Smith et al. 2002; Bahr et al. 2015). Originally these alien species were estimated to spread at a minimum rate of 250 m yr^{-1} (Rodgers & Cox 1999), although this is now considered to have been a gross underestimate (Coles et al. 2002; Smith et al. 2002). Since its introduction and release in 1974, *K.* clade B has resulted in phase-shifts across the bay by replacing native algae and corals with newly formed monocultures of this alien alga over large areas of reef flat and slope (Coles et al. 2002; Smith et al. 2002). Likewise alien algal overgrowth is smothering live corals on patch reefs, resulting in a loss of biodiversity, changes in community structure of the reef fishes, and erosion of the physical structure of the reef (Smith et al. 2002). In response to the spread of ecological impacts associated with these alien invasive species, the State of Hawaiʻi Division of Aquatic Resources (DAR) and The Nature Conservancy (TNC) have undertaken manual removal efforts using suction-assisted divers supported by the "supersucker" barges. The "supersucker" teams selectively remove invasive algae species from reefs with sorters on the surface looking for any native species accidentally removed from the reef. Materials that are sucked up through the pumps are sorted on the deck of the barge and any non-alien algae species are returned to the reef immediately. The alien algae is bagged and given to organic farmers who use it as natural fertilizer. However removal of alien algae by these teams is labor-intensive and only effective if the algae do not regrow, which was happening within roughly a year in the initial supersucker trials (DAR & TNC pers. comm.). Thus, the long-term solution proposed for areas where invasive algae have begun to overgrow and smother native corals has been to increase the population of native herbivores such as grazing urchins (Conklin and Smith 2005; Stimson et al. 2007; Weis & Butler, 2009).

Biocontrol agents have been the topic of much debate due to infamous cases during which their introduction lead to their subsequent invasion (Howarth 1983; Simberloff & Stiling, 1996). Introduced biolcontrol agents, which turned invasive, have wreaked irreversible damage to many host ecosystems (Howarth 1991). Notable instances of failed biocontrol efforts in Hawaiʻi include the introduction of the Rosy Wolf Snail (Howarth 1985, Holland et al. 2012), as well as the Indian Mongoose (Simberloff et al. 2000; Godwin et al. 2006). Indeed there are precious few examples of successful biocontrol efforts with alien species (Howarth 1983; Godwin et al. 2006). Many now argue that if biocontrol agents are to be used at all, they should

be native to the ecosystem being targeted (Howarth 1985). In addition to eliminating the likelihood that alien biocontrol agents become pests in a novel environment, it has been documented that native grazers suppress the establishment of exotic plants better than the introduction of exotic grazers (Parker et al. 2006; Kimbro et al. 2013).

Following on that logic, experiments with native sea urchins have demonstrated that *T. gratilla* have reduced the biomass of the invasive *Kappaphycus* spp. within enclosure areas on the reef where alien algae were abundant (Conklin & Smith 2005). Urchins are an important part of the macro-grazing fauna on many tropical reefs, including those in Hawai‘i (Chiappone et al. 2002; Alves et al. 2003; Mumby et al. 2006; Stimson et al. 2007; Sandin et al. 2010; Valentines & Edgar, 2010). Although some urchins show dietary preferences in laboratory studies (e.g., Stimson et al. 2007; Seymour et al. 2013), others appear to be generalist herbivores that will graze on just about any algae or sea grass made available (e.g., Vaïtilington et al. 2003; Dworjanyn et al. 2007). Other potential biocontrol agents, such as fish (acanthurids and scarids) exhibit a relatively low degree of preference for the invasive algae, are far more motile, and are highly prized by local fishermen, making it difficult to rely on herbivorous fishes as a potential mechanism of biocontrol (Conklin & Smith 2005). Urchins therefore make an obvious choice for a variety of algal biocontrol efforts because of their generalist feeding behavior and limited vagility as adults, coupled with the high dispersal potential and the ubiquity of habitat that has allowed a number of tropical urchins to successfully colonize reefs across the globe (Lessios et al. 2003; Seymour et al. 2013). Likewise, *T. gratilla* was historically abundant in Kāne‘ohe Bay (Ogden et al. 1989; Thomas 1994), but for unknown reasons has become rare since the 1990s (Stimson et al. 2007; Bahr et al. 2015).

Studies on the ecological impacts of natural outbreaks of *T. gratilla* corroborated the efficiency with which these urchins can significantly reduce the abundance of algae (Valentine & Edgar 2010). Due to their limited movement as adults and their voracious appetite for a wide variety of algae and seagrasses, *T. gratilla* has been recommended as the best species for use as a biological control agent in Kāne‘ohe Bay (Conklin and Smith 2005; Stimson et al. 2007). Since 2010, DAR has been culturing juvenile *T. gratilla* for outplanting as herbivorous biocontrol agents to prevent regrowth of algal biomass once the alien algae have been manually removed from patch reefs in Kāne‘ohe Bay (Gibo et al. 2012). This study set out to investigate if tank bred urchins would eat the targeted alien algae species, and if so, to determine their potential

grazing rates. The project also examined if the urchins' growth could be sustained on diets of non-native algae, and to what extent each alga facilitated growth of *T. gratilla*. Potential feeding preferences between the four alien algae were also evaluated. Lastly, urchins were caged in various habitats in order to elucidate post-transplant survival of tank bred juvenile urchins in the bay.

Currently, thousands of cultured urchins are outplanted at 20-25mm test diameter, but comparatively few were observed in subsequent surveys of urchin density on the reef (J. Blodgett, DAR, pers. comm.). A major motivation of this research was that it was unknown at that time whether the missing urchins were dying from transplant stress, starvation, being eaten by predators after outplanting, or simply moving into cryptic habitats at small sizes such that they were missing in subsequent surveys. Stimson et al. (2007) conducted feeding preference trials with large *T. gratilla* (8-9cm) and showed that feeding preferences were generally unchanged after 5 months on monospecific diets, except urchins that were maintained on *Padina sanctae-crucis* and showed enhanced preference in subsequent choice trials, whereas those maintained on *G. salicornia* tended to avoid it when offered five species from which to choose at the end of the trial. Further, Stimson et al. (2007) found that urchins offered a variety of algal species consume more per day than when limited to a single-species diet. This study expands on the previous work to elucidate patterns of the post-transport juvenile urchin survival, growth and diet preferences of lab cultured *T. gratilla* being outplanted in Kāne‘ohe Bay. Together, these studies will aid both State and conservation group efforts to control alien algal overgrowth of corals on reefs in Kāne‘ohe Bay and across Hawai‘i.

Materials and Methods

Study Animal:

The short-spined collector urchin *Tripneustes gratilla* received its common name from the habit of gathering fragments of coral rubble, rocks, or algae from the benthic environment as camouflage while it forages. Its body is predominantly black but often possesses a pentaradial bluish or reddish hue when its tube feet are retracted close to its body. Its spines are typically black, white or cream. This echinoid is relatively common in shallow waters (0-15m) across the Hawaiian archipelago (Kay 1994; Hoover, 2002). Natural densities of *T. gratilla* range from 2.9-4.4m^{-2}, placing it in the top three most abundant urchins in Hawai‘i (Ogden et al. 1989).

Although *T. gratilla* have shown significant dietary preferences for *Kappaphycus* spp. in controlled laboratory studies (Stimson et al. 2007), in the wild these urchins are a generalist herbivore that will graze on virtually any algae or sea grass available (Vaïtilington et al. 2003; Dworjanyn et al. 2007; Stimson et al. 2007). The generalist diet and habitat requirements of *T. gratilla* coupled with high dispersive potential have resulted in an extremely wide, pantropical distribution (Lessios et al. 2003). It should be noted that *T. gratilla* was formerly a native resident of Kāneʻohe Bay. *Tripneustes gratilla* was once thought to be one of the most abundant urchin species within Kāneʻohe Bay (Edmonson, 1946; Alender, 1964; Banner & Bailey 1970; Kay, 1994). Conversely, *T. gratilla* is now relatively rare and does not contribute significantly to herbivory on reefs within the bay (Conklin and Smith, 2005; Stimson and Conklin, 2008). However, historical outbreaks of the native alga *Dictyospaeria* were thought to be controlled by *T. gratilla* because growth accumulated mostly in calm waters of the bay where the urchin was rare (Banner & Bailey 1970). Hence, there is considerable interest from both State and local conservation groups to replenish the natural population of *T. gratilla* in the bay and enhance natural herbivory to control these invasive alien algal species.

Tripneustes gratilla were provided by the DAR urchin hatchery as juveniles. We arbitrarily placed urchins in three non-overlapping size classes: small (17.5-22.5mm maximum test diameter), medium (29.8-43.8mm), and large (45.1-65.1mm) that were then used for each of the experimental trials outlined below. Prior to the experiments, medium and large urchin size classes were raised on diets of all four alien algae. Small urchins were not fed before trials, but instead were placed directly into experiments within a few days of arriving from the hatchery. Therefore, urchins were starved 3-5 days prior to each experiment.

Algae:

We chose the four most common species of alien invasive algae found on the patch reefs of Kāneʻohe Bay: *Acanthophora spicifera, Gracilaria salicornia*, *Eucheuma denticulatum,* and *Kappaphycus* clade B. *Kappaphycus* clade B (formerly identified as *Kappaphycus alvarezii, K. striatum,* or *Eucheuma striatum*) and *Eucheuma denticulatum* were intentionally introduced from the Philippines to Kāneʻohe Bay September 1974 by researchers from the University of Hawaiʻi for scientific research (Doty 1971; Doty 1977); fragments apparently drifted away from test sites on the north reef of Moku O Loʻe (Coconut Island) and were also collected and transplanted around the bay by local residents for personal cultivation

(Russel, 1983; Batibasaga et al. 2003; Weis & Butler, 2009). Despite having observed vegetative propagules being released, researchers reported that such propagules were incapable of dispersing over deep water or finding suitable hollows on which to settle (Doty, 1977). This oversight led to the documented proliferation of new eucheumatoid colonies upon the introduction of the algae to test sites around Coconut Island (Doty, 1977). Likewise, the intentional introduction of *Gracilaria salicornia* by the same researchers to Kāneʻohe Bay occurred September of 1978, specifically for experimental aquaculture aimed at the development of commercial agar production (reviewed by Rodgers & Cox, 1999; Smith et al. 2004). The idea of a commercial agar industry in Hawaiʻi has long since been abandoned, but the introduced *G. salicornia* has established and spread along the shores of Waikīkī and reefs in Kāneʻohe Bay.

In contrast to these intentional introductions, a fragment of *Acanthophora spicifera* was first documented in Pearl Harbor in the fall of 1952, and was believed to have been transported on the heavily fouled hull of the barge "Yon 146" which was towed to Oʻahu from Guam in 1950 (Doty, 1961). By February 1956, *A. spicifera* had been documented in Kāne'ohe Bay, making it the first documented accidental introduction to the Bay (Kohn, 1961; Coles et al. 2002). These invasive macrophytes became not only some of the most dominant benthic organisms, but they have also resulted in the most detrimental impacts to marine communities in the bay (Coles et al. 2002).

The four algae species had widespread distributions in the bay and were readily available for collection. *Acantohophora spicifera* and *G. salicornia* were easily collected nearly anywhere around Coconut Island and around the southern portion of Kāneʻohe Bay. The eucheumoids were consistently collected from patch reefs in the central portion of the Bay. We did not included native algae in this study because their abundance is so reduced in the Bay (Stimson et al. 2001; Conklin & Smith 2005; Stimson et al. 2007) that we could not collect enough for this experiment without impacting the remaining population.

Growth on Single-species Diet

Growth rates of *T. gratilla* were measured while on single-species diets of each *A. spicifera, G. salicornia, E. denticulatum* and *K. alverezii*. For each of the four algal species, three *T. gratilla* were housed in each of three 15 L replicate aerated flow-through tanks (~1-2 L/min). In order to monitor individual growth rates of urchins without marking the animals, each tank held a single urchin of each size class: small, medium, and large. For each treatment, algae were

provided *ad libitum* to reduce any resource competition, and all aquariums were cleaned twice a week during which freshly collected algae were provided to each tank. Urchin test diameter was measured using Vernier calipers (VWR) to the nearest tenth of a millimeter each week for a month. An analysis of covariance (ANCOVA) was employed to analyze growth, with initial urchin test size used as the covariate. Tukey's HSD post-hoc comparison was then performed to determine significance of pairwise differences in average growth rates of urchins on each algae diet (Fig. 1).

No-Choice Feeding Trials:

No-choice feeding trials provided juvenile *T. gratilla* in each treatment with only a single species of alga and measured differences in mean consumption among the treatments with different algal species. For each algal species, two urchins of the same size class were added to each of six replicate tanks (15L tanks with 1-2L/min flow rate, as above). Algae were blotted on paper towels to remove excess water and weighed before being placed in each tank. Urchins were allowed to graze for ~5 days and the amount of remaining algae was weighed as before to calculate the amount of each species consumed per urchin per day. In a few cases we stopped the experiment after the 4^{th} day because we did not want the urchins to consume more than half of the algae offered in any trial. Consumption rates (grams of algae per day) were then compared by analysis of variance (ANOVA). Tukey's HSD post-hoc comparison was used to identify significant differences between each of the algae (Fig. 2). The assumptions of Normality and homogeneity of variance of the data were tested using the Shapiro-Wilks test and the Levene's test, respectively. The null hypothesis for the Shapiro-Wilks test was that the data were Normally distributed; therefore, p-values less than 0.05 suggested that the data were not Normally distributed. For the small size class in the no-choice feeding trials the data were Normally distributed (Shapiro-Wilk, $W = 0.96$, $p = 0.82$). The data from the medium cohort from the no-choice trial was also normally distributed (Shapiro-Wilk, $W = 0.90$, $p = 0.28$). However, the data from the large urchins of the no-choice feeding trial were only marginally non-normal (Shapiro-Wilk, $W = 0.80$, $p = 0.042$). To test the homogeneity of variance among our feeding trial data, the Levene's test was employed. The null hypothesis for the Levene's test was that the variances are homogenous. For the urchins in the no-choice feeding trials, the data passed the homogeneity test (Levene's test, $F(3, 44) = 0.56$, $p = 0.64$).

Choice Feeding Trials:

The choice feeding experiment provided all four species of algae, in equivalent amounts, simultaneously to urchins. As with no-choice experiments above, each algal species was blotted and weighed before being introduced to the experimental tanks. For this assay, larger tanks (80L, ~4L/min flow-through) were used to allow room to separate algae into the four quadrants of the experimental tank. Four urchins were then introduced to the middle of the tank and allowed to graze at will for ~7 days. Again, if any species of alga became low relative to the others (less than half the initial amount), we stopped the experiment a day early to avoid biasing results. At the end of each experiment, algae were removed, blotted and weighed as previously to calculate the amount of algae consumed per urchin per day for each species.

For each choice and no-choice feeding trials, small urchins were provided with ~100g of algae initially, whereas medium and large urchins were offered a starting biomass of ~150g of algae. To account for any growth or decline of the algae not attributed to urchin grazing during the experiment, both choice and no-choice experiment tanks had a divider such that one half of the tank housed experimental algae and urchins whereas the other side housed only equivalent amounts of algae to serve as a no urchin control. The consumption rates of algae were then calculated as:

$$\text{Consumption} = (A_i\ (AC_f / AC_i) - A_f)$$

Where A_i and A_f were the initial and final blotted masses of algae subject to grazing by urchins; while AC_i and AC_f were the initial and final masses of the algae in the no-urchin control tanks. This equation was used to account for growth of algae over the course of the experiment, but can also account for any unexpected decline in algal biomass unrelated to the grazing trial (Dworjanyn et al. 2007; Seymour et al. 2013). Because all species of algae were provided simultaneously during choice feeding trials, the consumption of one species was affected by the consumption of the others, therefore the assumption of independence required to perform an ANOVA was violated. Consequently, choice feeding preference assays were analyzed using a non-parametric Friedman's rank test, and both parametric and non-parametric analyses are congruent. Relative consumption rates of each algal species were reported (Fig. 3) and ranked. Nevertheless, due to the lack of post-hoc pairwise comparison for Friedman's rank test, a Tukey's HSD post-hoc comparison was used to identify significant differences between each of the algae. Again, to test the data's distribution for Normality and homogeneity of variance the Shapiro-Wilk and Levene's test were used. The data from the small urchins of the choice

feeding trials failed the normality test (Shapiro-Wilk, W = 0.86, p = 0.0036). However, the data for the medium and large sized urchins of the choice feeding trials both passed the normality test (Shapiro-Wilk, W = 0.97, p = 0.27, and W = 0.96, p = 0.17, respectively). For the small urchins of the choice feeding trial, the data passed the homogeneity test (Levene's test, F(3,20) = 2.13, p = 0.13). The data from the medium urchins in the choice feeding trial fail to reject the null hypothesis (Levene's test, F(3,36) = 0.32, p = 0.81). The data from the cohort of large urchins in the choice feeding trials also passed the test for equality of variances (Levene's test, F(3, 40) = 1.98, p = 0.13).

Field Caging Experiment:

Cages measuring roughly 50x50x75cm were constructed from 1cm^2 galvanized chicken wire mesh. We constructed both open and closed cages. For closed cages, the mesh extended across all sides including the tops to prevent urchins from being able to crawl out and prevent access by fishes on the reef. In contrast, the sides of the open cages end with back-folded edges and no top to minimize escape of the juvenile urchins from the cage, but still allow open access of predatory fishes. Initial trials with urchins caged in seawater tables indicated that this back-folded edge design (approximating an upside down U) was the most effective for open cages, but urchins still escaped the cages at the rate of 1-2 animals per week. Urchins ranged from 18-22mm at the start of the experiment. Cages were filled with *G. salicornia* to provide the juvenile urchins with food and a place to hide, because our initial aquarium trials revealed that urchins were far more likely to escape the open cages in the absence of hiding spots and food in the cage. In the absence of any cover or food, open cages were frequently empty within 24 hours in our water table trials (data not shown).

Cages were placed at 6 sites across three habitats, with four cages, three open and one closed control, at each site. 251 urchins were used during these caging experiments. Three habitats surrounding Coconut Island (map in supplementary materials) were selected to mimic the conditions on the reef to which urchins are being currently outplanted: a protected lagoon, a shallow back-reef and a fore-reef slope each at 1-3m depth. The protected lagoon had low coral cover, high alien algal cover and minimal water flow, whereas both the back-reef and fore-reef sites had high coral cover, relatively low alien algal cover and relatively high water flow. Each cage was checked three times a week for 30 days to count surviving urchins as well as replenish consumed algae. All studies reported here were conducted under the State of Hawai‘i,

Department of Land and Natural Resources, Division of Aquatic Resources Special Activity Permits sap#2012-63 and SAP#2013-47. Survivorship between treatments was compared using the Kaplan-Meier product-limit method for fitting survivorship curves and comparison by Log-rank (Forsman et al. 2006), and Wilcoxon non-parametric tests. These statistical tests were done using JMP Pro 11.

Results

Growth on Single-species Diet:

Growth rates of *T. gratilla*, measured as maximum test diameter (mm), were significantly affected by fixed algal diets (ANCOVA, initial urchin size as covariate, $F_{(5,84)} = 10.80$, $p < 0.001$, Fig. 1). Urchins that fed exclusively on diets of either *G. salicornia* or *K.* clade B grew at significantly ($p = 0.001$ and $p = 0.009$, respectively, Tukey HSD) higher rates (1.58 ± 0.14 and 1.69 ± 0.14mm/week TD (Test Diameter), respectively) than those urchins that fed on a diet of *E. denticulatum*. Urchins that fed on a diet of *E. denticulatum* had the lowest growth rates (0.97 ± 0.14mm/week TD) out of the four assays, though not significantly lower to urchins on a diet of *A. spicifera* (1.23 ± 0.11 mm/week TD).

No-choice Feeding Trials:

When presented with no choice, urchins consumed different species of algae at different rates, but the effect varied by urchin size (Fig. 2). On average, large urchins ate *G. salicornia* at the highest rate of all algal species offered (15.24 ± 0.001 g.day^{-1}), and *K.* clade B at the lowest rate (12.43 ± 1.51 g.day^{-1}) although these trends were not significant (ANOVA, $F_{(3,12)} = 1.94$, $p = 1.78$, Fig 2C). Medium sized urchins showed similar trends, but with significant differences in the amounts of algae they consumed on a daily basis (ANOVA, $F_{(3,12)} = 8.49$, $p < 0.05$, Fig. 2B). For the medium size class, *Gracilaria salicornia* was eaten at a significantly higher rate (12.60 ± 0.08 g.day^{-1}) than either *A. spicifera* (10.33 ± 0.36 g.day^{-1}) or *E. denticulatum* (9.35 ± 0.90 g.day^{-1}) ($p = 0.034$ and $p = 0.003$, respectively, Tukey HSD); *K.* clade B was also eaten at a higher rate (11.87 ± 0.27 g.day^{-1}) than *E.denticulatum* ($p = 0.018$, Tukey HSD), but not *A. spicifera* ($p > 0.05$, Tukey HSD). Among the small collector urchins, feeding rate patterns were comperable to those of the medium urchins, but with more significant disparities among algal species, (ANOVA, $F_{(3,12)} = 51.30$, $p < 0.001$, Fig. 2A). Small urchins offered only *G. salicornia* had a significantly higher mean consumption rate (6.08 ± 0.19 g.day^{-1}) than any other algal assay in

the non-choice feeding trial ($p < 0.05$ for each pairwise comparison, Tukey HSD). Small urchins likewise consumed *Eucheuma denticulatum* at the lowest rate (2.32 ± 0.39 g.day^{-1}), which was significantly lower than both *A. spicifera* and *K.* clade B ($p < 0.05$, Tukey HSD).

Choice Feeding Trials:

Feeding trials in which urchins were offered multiple species of algae simultaneously revealed different patterns than those observed in the non-choice feeding assays. Small urchins significantly preferred to feed on *A. spicifera* than any of the other three available algae species (Friedman's rank test, $p < 0.05$; $p \leq 0.01$, Tukey HSD, Fig. 3A). Small urchins did not display any patterns of preference among *G. salicornia*, *E denticulatum* or *K.* clade B ($p > 0.05$, Tukey HSD). Likewise medium urchins showed a significant preference (Friedman's rank tests, $p < 0.001$, Fig. 3B) for *A. spicifera* over *G. salicornia* and *K.* clade B ($p < 0.05$ and $p < 0.001$, respectively, Tukey HSD), as it was consumed at the highest rate (3.69 ± 0.21 g.day^{-1}), whereas *G. salicornia* and *E. denticulatum* were consumed at intermediate rates (0.95 ± 0.1 and 1.19 ± 0.13 g.day^{-1}, respectively). In contrast, medium urchins consumed *K.* clade B at the lowest rate (0.51 ± 0.10 g.day^{-1}). Unlike the small and medium urchins, which avoided *K.* clade B in the choice trials, large urchins exhibited significant preferences for both *A. spicifera* and *K.* clade B (4.30 ± 0.09 g.day^{-1} and 4.31 ± 0.14 g.day^{-1}, respectively*)* in the choice feeding trials (Freidman's rank test, $P < 0.001$, Fig. 3C). Large urchins significantly preferred *A. spicifera* to both *G. salicornia* and *E. denticulatum* ($p < 0.0001$ and $p < 0.001$, respectively, Tukey HSD). *Kappaphycus* clade B was also significantly preferred to *G. salicornia* and *E. denticulatum* ($p < 0.01$ and $p < 0.05$, respectively, Tukey HSD). Whereas *G. salicornia* and *E. denticulatum* were consumed at intermediate rates by small and medium urchins, these species tend to be avoided by the large urchins (3.11 ± 0.20 g.day^{-1} and 3.20 ± 0.20 g.day^{-1}, respectively) when given a choice of algae on which to feed (Fig 3).

Caging Experiment:

Considerable differences in urchin survivorship among the three habitats that they were caged in were found to be significant (Table 1). On the reef flat, 84.4% of urchins deployed in closed cages remained and 75% of urchins remained in the open cages after 29 days (Fig. 4A). Urchins caged in the lagoon had a substantially higher rate of loss than those on the reef flat,

with only 56.3% of urchins remaining after 29 days, regardless of cage type (Fig. 4B). After 29 days, 55.2% of urchins remained in closed cages, but only 20% survived in the open cages, giving urchins deployed on the reef slope the highest rate of loss (Fig. 4C).

Discussion

Increasing the abundance of native grazers would not only control and remove current alien invasive algae species, but it would also serve to increase the degree of biotic resistance to novel invasive species (Kimbro et al. 2013). Consistent with previous studies (e.g., Lawrence & Agatsuma 2001; Seymour et al. 2013), *Tripneustes gratilla* is a generalist herbivore that managed to grow on every species of algae tested, and fed on all algal species offered to them without exception. However, *T. gratilla* did not interact with every species of algae indiscriminately. The urchins experienced variable growth depending on the diet they fed on. They did not feed on all rhodophytes at the same rate, and even exhibited preferences between the available species when presented with a choice of all four alien algae species. All of the algal diets supported growth, but the algae species that supported the highest growth rates were *G. salicornia* and *K.* clade B*,* whilst urchins that fed solely on *E. denticulatum* had the lowest growth rates, and *A. spicifera* sustained intermediate growth (Fig. 1). The reason for differential growth was not investigated, but could possibly be attributed to variation in nutritional content, consumption rate, digestibility or assimilation efficiency (Sterner & Hessen 1994). For example, growth rates may be higher on *G. salicornia* than *A. spicifera* because *Gracilaria* contains more protein than does *Acanthophora* (McDermid et al. 2007). Likewise, the low growth rate of urchins on a diet of *E. denticulatum* could result from the comparatively high dietary fiber of this alga, a compound that resists digestion and lowers assimilation efficiency (McDermid et al. 2005).

As with consumption rates, feeding preferences were also observed to vary among size classes (Fig. 3). The differences in feeding preference and consumption rates could translate into urchins of different size classes having differential impacts on alien algae, and argue that biocontrol efficiency could be increased by outplanting urchins of the correct size class for the dominant algal species to be controlled. For example, urchins of all size classes consumed *A. spicifera* preferentially in choice experiments (Fig. 3). *Acanthophora spicifera* is known to be

consumed by other native grazers (Wylie & Paul, 1988; Russell & Balazs 1994), but the effects of alien algal ingestion on the diets of native herbivores remains unknown (Smith et al. 2004). Our results show a greater disparity of preference among smaller than larger urchins (Fig. 3), suggesting that the role of alien algae in the diets of native grazers may vary ontogenetically. Nevertheless, small urchins showed a significant preference for *A. spicifera* relative to all other species, whereas larger urchins showed a higher affinity for both *A. spicifera* and *K.* clade B. Despite being a preferred species in all choice feeding trials, urchins did not seem to grow at a significantly different rate (1.23 ± 0.11mm/week test diameter) when fed only *A. spicifera* relative to any other algal diet (Fig. 1). In fact, growth rates appeared maximal on a diet of *G. salicornia* which was less preferred in all choice feeding trials (Fig. 3).

Our results contrast those presented by Stimson et al. (2007), which focused solely on large adult urchins (7-8cm test diameter), and showed a significant preference of urchins for *Kappaphycus* spp. but without including *Eucheuma denticulatum* in the feeding trials. Here we find that consumption rates of each algal species vary by size class, and we see no significant differences among consumption rates of algal species for urchins in our largest size class (Fig. 2). Given the strong differences seen in diet preference among urchins of different size classes, the discrepancy between our results and those of Stimson et al. (2007), could result from a continued change in diet preference and their use of significantly larger urchins than used in our feeding experiments. Among medium urchins, however, significantly more *G. salicornia* and *K.* clade B were consumed, and the smallest urchins consumed significantly more *G. salicornia* than the other three species (Fig. 2). Although the general trend is similar among urchin size classes, the biggest differences among the consumption rates were seen in the smallest urchins, which consume slightly more than twice as much *G. salicornia* as *E. denticulatum* (Fig. 2A). However, as urchin test diameter increased, there was a reduction in the difference between feeding assays, until all four species were consumed at statistically indistinguishable rates among adult urchins in the no-choice trials; larger urchins eat more algae, and grazing rates become more homogeneous among the four species of red algae (Fig. 2). Algal palatability can be reduced by increased algal toughness in a range of herbivorous species (e.g., Peters et al. 2002). For some species of echinoderms, it has been documented that larger sizes (body diameter) are associated with greater jaw strength (Ellers & Telford, 1990). Thus, it may be that larger urchins

are better able to masticate a wider variety of algae, including species with larger and tougher thalli, such as *E. denticulatum* and *K.* clade B.

It is noteworthy that despite the widespread distribution of *T. gratilla*, caged juvenile collector urchins did not fare equally well in every environment. The caging experiment highlighted significant differences in survivorship among potential outplanting locations (Table 1). Urchins placed in high algal cover, comparatively low-flow lagoonal habitats fared poorly, with only ~50% survivorship (Fig. 4B). Survivorship was equal for animals in both open and closed cages in the lagoon, and the removal of empty tests (clear evidence of mortality) accounted for all but a couple animals by the end of the month deployment. Thus, we are confident that the decline in urchin number in the lagoon was a result of low survival as opposed to predation or escape. Urchins caged on the reef flat consistently had the highest overall survivorship rates, with nearly 80% of urchins remaining at the end of the experiment. In these sites, we found only a single urchin test in any cage, and we had a few urchins somehow escape the closed cages in our initial water table trials, so we cannot be sure of whether the missing urchins died or escaped, but the high overall survival in both open and closed cages provides evidence that placement of urchins in these habitats is likely to increase the population of herbivorous urchins over time. In contrast, juvenile urchins placed on the reef slope suffered mortality rates as high as 80% in open cages (Fig. 4C). Even in closed cages, survival over the course of the experiment was only slightly above 50%, and again we recovered only 25 total tests in the cages over the course of the experiment. This seems likely to be a result of predation by reef fishes because we see a much more dramatic decline on the deeper water reef slope than in the shallows of the reef flats (Fig. 4). The decline of urchins in the closed cages on the reef slope below what is seen in open cages on the reef flat is somewhat puzzling. However, we noted during the experiment that the saddleback wrasse, *Thalassoma duperrey*, was particularly abundant around the urchin cages along the reef slope. Wrasses have been documented harassing juvenile *T. gratilla* urchins (Dafni & Tobo, 1987), and in a couple of cases, fish were even found to have somehow squeezed themselves inside one of the closed cages along the reef slope and were subsequently trapped in the cage on the next day. Although the mesh size was intended to exclude predatory fish, it is possible that wrasses small enough to fit through 1 cm^2 mesh may have been responsible for the decline of urchins in closed cages along the reef slope,

and the loss of 80% of animals from open cages within a month suggests that outplanting urchins into deeper waters of the bay is simply generating a feeding station.

Conclusion

The estimated overall productivity of these alien invasive eucheumatoids in Kāne‘ohe Bay is 20.8 tonnes dry wt/ha/yr, which translates to 5.7 g dry wt/m^2/day (Glenn & Doty, 1990). Given this approximation of productivity and the data compiled on consumption rates, a rough estimate of the ideal urchin density can be derived. Here we find that a large urchin (45-65mm TD) could graze ~7.5g of alien algae per day when presented with a mixed diet. This back-of-the-envelope calculation suggests that the grazing rate of one adult urchin/m^2 may be just about equal to the predicted growth rates of these algae. Additionally, our caging experiments indicate that mortality rates for juvenile urchins in open cages on the reef flat are on the order of 25%. Thus, a target density of two urchins/m^2 is recommended to overcome growth and reduce the biomass of alien algae if urchin grazing is to be effective for biocontrol. Given the high mortality rates for juvenile urchins on the deeper reef slope and the protected lagoon habitats, it is not advisable to invest the effort to culture and outplant juvenile urchins in either environment. Although there is variability in growth rates, juvenile urchins tend to grow quickly and the largest size class of urchins we tested showed no significant preferences among any of the target alien invasive algal species. Given that current efforts by conservation groups aim to manually remove alien algae and then outplant native urchins to the reef flats, where survivorship was high, this study increases confidence that intentional outplanting of juvenile urchins is likely to be an effective means of biocontrol for these invasive alien algae.

Table 1. Analysis of Kaplan-Meier survivorship curves for the urchin caging experiment: A comparison of survivorship distributions between open and closed cages placed in the lagoon, the reef flat and the reef slope.

		df	Log rank		Wilcoxon		Verdict
			Statistic	*P*	Statistic	*P*	
I. Between all treatments		5	47.15	<0.0001	41.36	<0.0001	
II. Between open cages		2	38.62	<0.0001	33.59	<0.0001	
	R vs L	1	4.43	0.0352	3.17	0.0750	R ≥ L
	R vs S	1	37.79	<0.0001	32.66	<0.0001	R > S
	S vs L	1	13.01	0.0003	12.06	0.0005	L > S
III. Between closed cages		2	6.73	0.0346	6.16	0.0460	
	R vs L	1	4.08	0.0434	3.62	0.0571	R ≥ L
	R vs S	1	6.13	0.0133	5.73	0.0166	R > S
	S vs L	1	0.05	0.8315	0.15	0.6959	ns

Abbreviations: R = reef flat, S = reef slope, L = lagoon, ns = not significant.

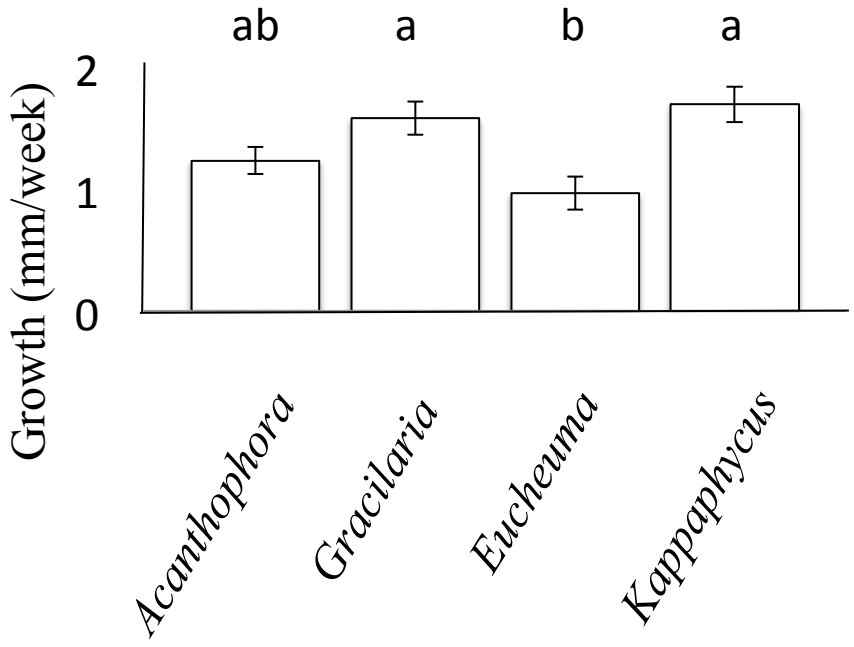


Figure 1. Urchin growth: Mean weekly growth rate (mm/week ± SE) of *Tripneustes gratilla* on non-choice diets of algae (*Acanthophora spicifera n* = 26, *Gracilaria salicornia n* = 28, *Eucheuma denticulatum n* = 27, or *Kappaphycus clade B n* = 9) reared in aquaria over a 4-week period. Note letters identify significant subsets ($p < 0.05$, Tukey HSD).

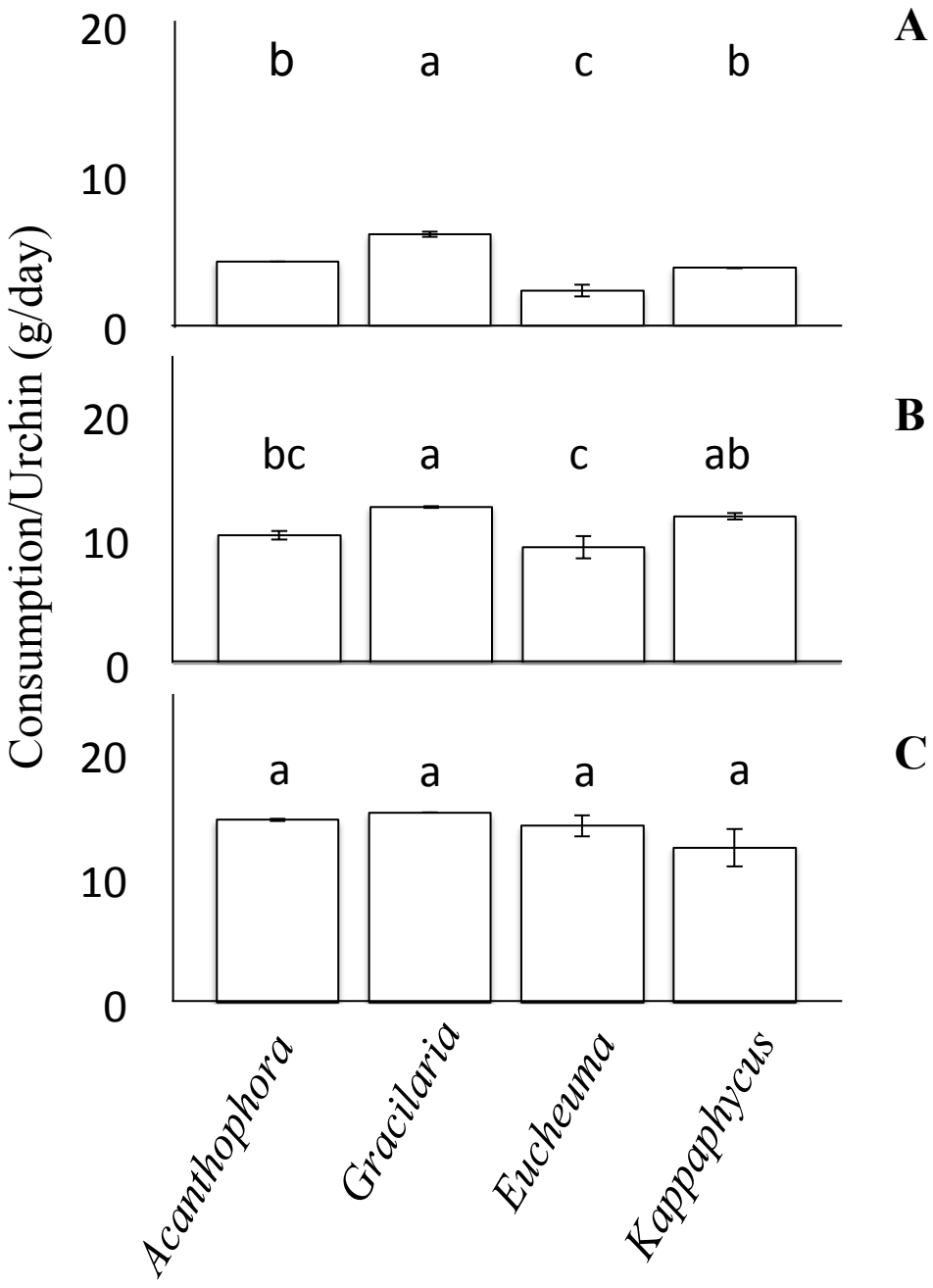


Figure 2. No-choice feeding trials: Consumption rates (g/day ± SE) by *Tripneustes gratilla* during non-choice feeding trials of algae (*Acanthophora spicifera, Gracilaria salicornia, Eucheuma denticulatum, or Kappaphycus clade B*). (A) Small. (B) Medium. (C) Large Urchins. Note letters identify significant subsets ($p < 0.05$ Tukey HSD post-hoc pairwise comparison). For each diet of each size cohort $n = 4$.

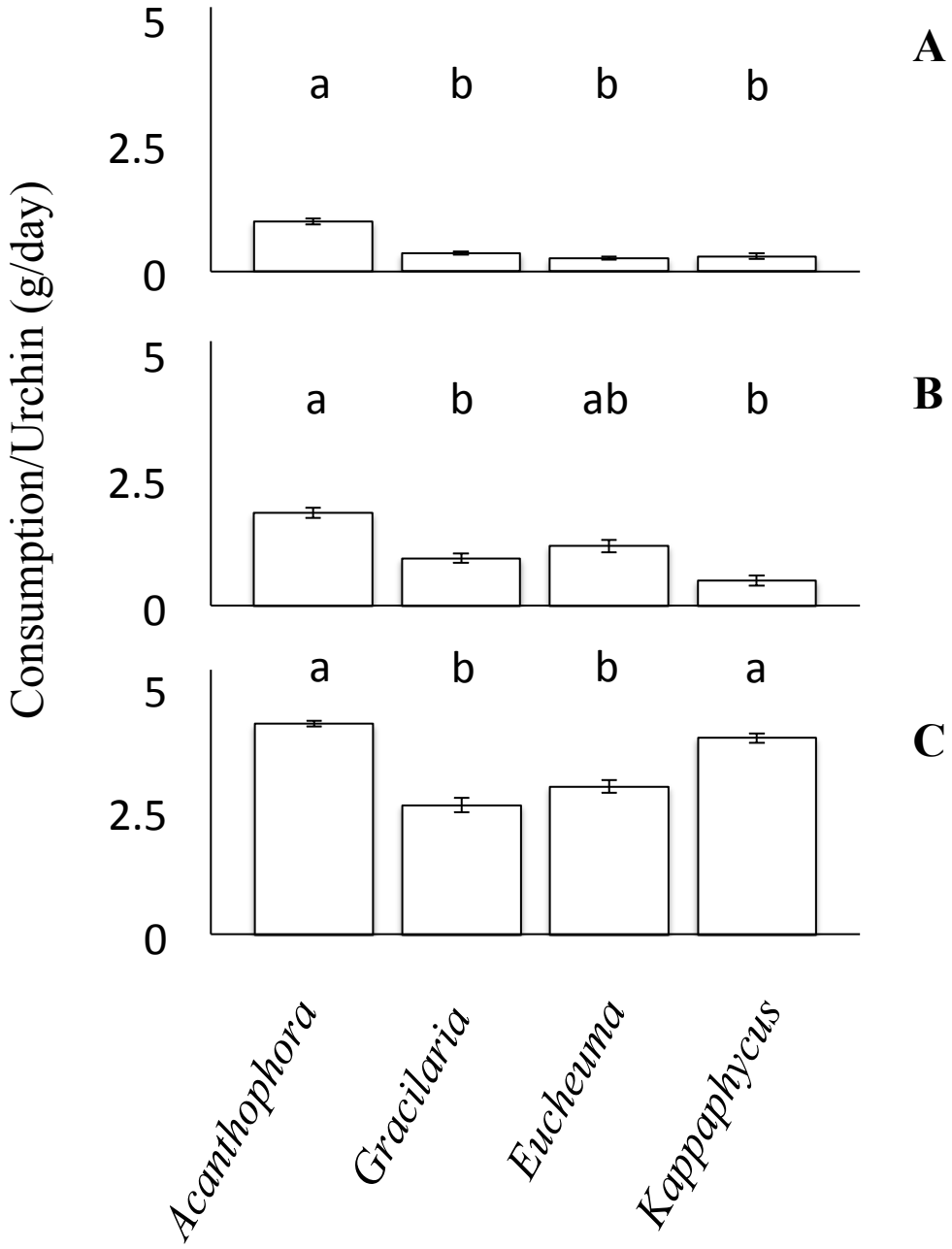


Figure 3. Choice feeding trials: Consumption rates (g/day ± SE) of *Tripneustes gratilla* during three choice feeding trials of algae (*Acanthophora spicifera, Gracilaria Salicornia, Eucheuma denticulatum, and Kappaphycus clade B*). (A) Small, *n* = 6. (B) Medium, *n* = 10. (C) Large, *n* = 11.

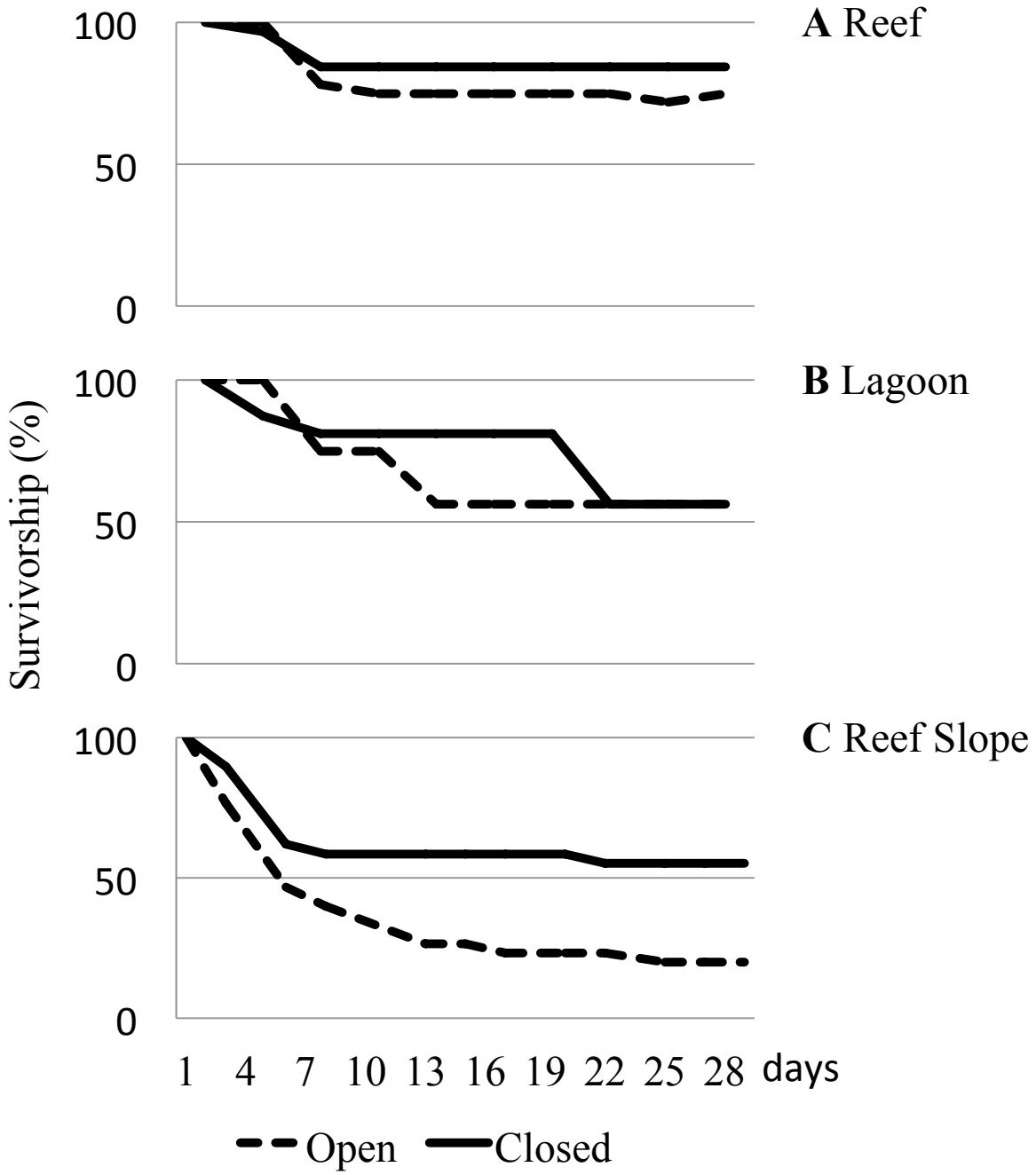


Figure 4. Caged urchin survivorship: Survivorship (%) curves reported for urchins deployed in open and closed cages in various underwater habitats for a month. (A) Reef. (B) Lagoon. (C) Reef Slope. Two hundred and fifty-one urchins were used across 6 sites.

CHAPTER 3: CONTRIBUTIONS TO BIOCONTROL OF INVASIVE ALGAE

Invasive algae removal strategies

Marine ecosystems around the world have become plagued by alien invasive algae (Anderson, 2007; Williams and Smith, 2007). This problem has had researchers and environmental managers conceiving, testing and developing a vast array of algal removal strategies. Approaches have included: preventative ballast water treatment with high voltage pulses or the use of ozone and UV radiation to kill invasive algae before discharging it from ships (Sun et al. 2011; Wu et al. 2011), the use of algicides and biocides such as Reglone A, potassium permanganate, sodium hypochlorite or household bleach, Simazine, titanium dioxide and chelated copper compounds (Lam et al. 1995; Anderson, 2005; Peller et a. 2007; Gumbo et al. 2008; Jellyman et al. 2011), burial of algal thalli (Glasby et al. 2005a), the administration of salt in high concentrations (Glasby et al. 2005b), heat treatment of thalli and gametophytes (Wotton et al. 2004), freshwater exposure (Theil et al. 2007), manual and mechanical removal through the use of brushes and benthic vacuums (Smith et al. 2004; Ceccherelli & Piazzi, 2005; Conklin & Smith, 2005; Weijerman et al. 2008; Kamalakannan et al. 2014; Marks et al. 2017), and biological control using agents such as bacteria (Gumbo et al. 2008), sea slugs (Thibaut & Meinesz, 2000), urchins (Conklin & Smith, 2005; Sumi & Scheibling, 2005) and fish (Weijerman et al. 2008). These methods have exhibited varying levels of success, with no single tactic providing a universal solution to invasive algae. Management efforts must be tailored to the pest as well as the affected ecosystem to effectively control or eradicate the invasive species.

Contributions and Implications to Invasive Algae Management

For alien algae invasions that occur on coral reefs, as is the case in Kāne'ohe Bay, the intruding macrophytes cannot be managed in isolation. This bay hosts a complex coral community of high conservation value. The seaweeds targeted in this study grow in close proximity to live corals; sometimes appearing interlaced with native Scleractinian corals. Maximizing algal removal while minimizing impacts to native species renders strategies that alter water quality (salinity, temperature, pH and chemical composition) untenable. The use of algicides would likely cause harm to the corals' symbionts, as the chemicals are often designed to damage photosynthetic pigments. Other biocides and chemicals should be avoided on coral reef communities due to the

chemicals' lack of environmental sensitivity (Godwin et al. 2006). Heat treatments would be far too time consuming to treat large areas and would cause unavoidable non-target effects to nearby species. Burying algae under sediment is very disruptive to the benthos and would be destructive to corals; furthermore it has had low success rates against invasive algae in the Mediterranean (Glasby et al. 2005a). Large amounts of invasive algae have been manually removed in Kāne'ohe Bay through the coordinated efforts of volunteers (Smith et al. 2004) and dive teams using surface supported mechanical suction pumps (Conklin & Smith, 2005; Godwin et al. 2006; Marks et al. 2017). Manual removal is a popular strategy against invasive algae as it is target specific and therefore assumed to produce the fewest detrimental side effects to the environment among all potential management strategies (Thresher & Kuris, 2004). However, manual removal is extremely labor intensive, requiring many person-hours/m^2 to fully clear sections of benthos (Smith et al. 2004; Conklin & Smith, 2005; Weijerman et al. 2008). Removing all of the microscopic holdfast cells and propagules is impossible (Godwin et al. 2006), explaining why invasive algae have recovered in as little as two months from intensive manual removal efforts (Smith et al. 2004; Conklin & Smith, 2005; Weijerman et al. 2008). Manual removal of *Kappaphycus alvarezii* was reported as an inefficient (even counterproductive) control strategy in the Gulf of Mannar, India, as the alga was able to recover from residual holdfasts and dispersed propagules that settled and then grew on nearby reefs (Kamalakannan et al. 2014). That being said, the use of floating curtains has been suggested as a remedy to reduce the dispersal of algae fragments and other propagules during manual removal efforts (2007). Still, manual removal remains an effective way to quickly remove large amounts of algal biomass, although few examples of successful invasive algae eradication have been attributed to this approach (Miller et al. 2004). Regular manual removal is also very costly and consequently an unsustainable treatment to a problem that is likely to outlast the effort to manage it. Conversely, biological controls have potential for long-term management of invasive species on Hawaiian reefs (Coles et al. 2002). Previous studies have demonstrated that *Tripneustes gratilla* was able to reduce the biomass of *Kappaphycus spp*. in small enclosures (Conklin & Smith, 2005). Stimson et al. (2007) suggested that the collector urchin's slow movement and opportunistic feeding behavior would provide complementary grazing pressure against invasive algae species, thus advocating for its potential use in invasive algae management. Furthermore, farmed collector urchins incorporate invasive algae into their diets at similar rates to wild collector

urchins, and native *T. gratilla* are good biological control candidates against *Acanthopora spicifera* and *Gracilaria salicornia* (Van Heukelem, 2016). Reports of *T. gratilla* outbreaks attest to the urchin's ability to dramatically reduce algal biomass while having no impact on coral cover or density (Valentine & Edgar, 2010).

This investigation examined the collector urchins feeding preference, growth and survivorship. Findings were congruent with Van Heukelem (2016) with respect to collector urchin's affinity for *A. spicifera*. However, as the urchins grow they seem to develop a more generalist feeding behavior, as implied by Stimson et al. (2007). The urchins were able to grow on all regimens provided, demonstrating their ability to successfully incorporate invasive algal species in their diet. Analysis of caged urchins revealed significantly higher rates of survival on the reef flats compared to the deeper reef slope and the lagoon habitats. Management guidelines derived from these findings would encourage deployment efforts to focus on reef flat habitats, and to consider variable feeding preferences for different urchin sizes as a reason for disparate algal removal rates in the field. Feeding preferences are also a potential tool for targeting different invasive algae species.

Because invasive algae have been established over large areas of O'ahu's reefs for decades, complete eradication is unlikely. Nonetheless, successful management is still achievable if the impacts of the invasive algae can be reduced to an economically and ecologically acceptable level (Anderson, 2007). This project can be incorporated into the larger body of research supporting the use of the native collector urchin as an augmentative biocontrol agent in Hawai'i (Coles et al. 2002; Conklin & Smith, 2005; Godwin et al. 2006; Stimson et al. 2007; Van Heukelem, 2016). Combining manual removal of alien macrophytes with biological control, using native collector urchins, could produce a synergistic interaction that would maximize the removal of invasive algae. However, manually removing the bulk of the seaweeds first would expedite elimination of the algal biomass. Subsequent increased grazing pressure, from deployed urchins, would serve to suppress alien algae and inhibit its resurgence. Augmenting the abundance of native browsers would not only increase the grazing pressure on invasive algae, it would reinforce an ecologically crippled ecosystem's herbivorous functional group (Stimson et al. 2001). A stronger community of grazers would also provide resilience to future invasive algae introductions.

Subsequent research directions

The completion of this project has spurred a variety of tangential research ideas revolving around the further development of biological control against invasive algae. Healthy functional groups, comprised of multiple species, tend to increase ecological resilience. By expanding the assemblage of biocontrol agents capable of targeting invasive algae, grazing pressure could be dramatically increased. Consequently, the development of another biological control agent may warrant consideration. The herbivorous green sea turtle, *Chelonia mydas*, is native to the Hawaiian Islands. Although *C. mydas* exhibit omnivorous tendencies as juveniles, their feeding behavior switches to a predominantly herbivorous regimen as they mature (Cardona et al. 2010). Reports have indicated that green sea turtles in the Hawaiian Archipelago have incorporated invasive algae into their diets, namely: *Hypnea musciformis, Acanthophora spicifera, Gracilaria salicornia, Eucheuma denticulatum, Kappaphycus alvarezii* and *K. striatum* (Russel & Balazs, 1994; Arthur & Balazs, 2008; Russel & Balazs, 2009). Perhaps most surprising was that three of these invasive species, *A. spicifera, H. musciformis* and *G. salicornia*, were the most common components found in *C. mydas*' diet (Russel & Balazs, 2009). Furthermore, *C. mydas* around the Hawai'i Institute of Marine Biology at Coconut Island can remove all visible traces of *G. Salicornia* (pers. obs.). In fact, the suppression of the *G. salicornia* was so thorough that none could be collected in the area for experiments. Therefore, *C. mydas* should be further studied for potential use as an augmentative biocontrol agent in the Hawaiian Island. Turtles hatcheries could be opened, conceptually similar to the urchin hatchery, as turtles farms have already demonstrated success in releasing thousands of *C. mydas* into the wild (Bell et al. 2005). Moreover, as the Hawaiian green turtle is classified as threatened under the U.S. endangered species act, these efforts would serve the dual purpose of fighting invasive species, while promoting recovery of a species of conservation concern.

The next potential research idea deals with the methods of propagation of invasive algae. *Kappaphycus alvarezii* has been reported to lack a sexual reproductive cycles in Kāne'ohe Bay, and is therefore assumed to reproduce exclusively via vegetative fragmentation (Rodgers & Cox, 1999; Conklin & Smith, 2005). Nevertheless, it would be interesting to test this empirically. Perhaps a genetic approach could be used to estimate rates of fragmentation or cloning across invasive algae meadows. This could provide conclusive evidence on whether the algae are only able to spread by fragmentation, or if sexual reproduction occurs at low frequency. Information

derived from such a study could shed light on the genetic structure of the algal outbreaks. Findings could also have implications for manual removal efforts. If fragmentations rates are high, then perhaps strategies for containing the alga should be developed before attempting further removal, as such disturbances could further spread invasive algae. Although data from such a project could prove insightful, results could be confounded if high rates of coalescence (fusing of fragments) occur among the targeted algae species (Santelices, 2004).

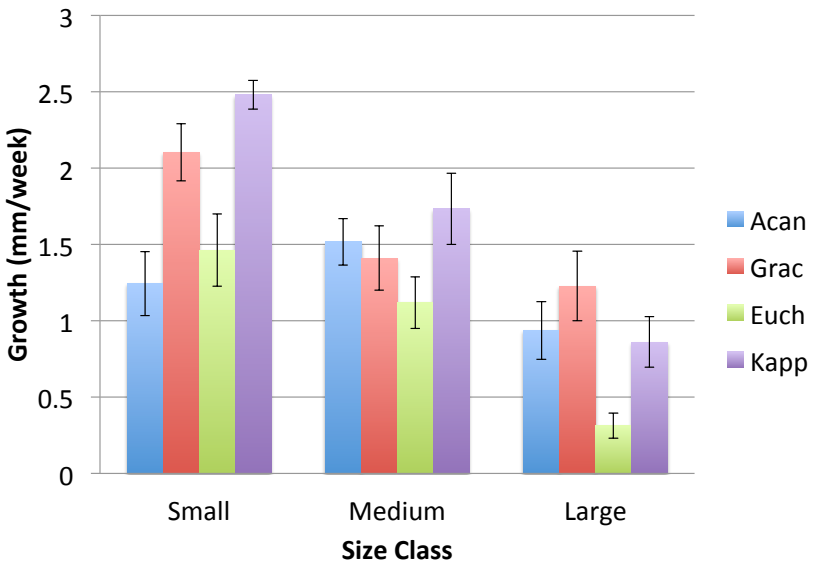
To support the ongoing efforts of native biocontrol release, an examination of artificial reproductive methods merits further attention. *Tripneustes gratilla* have seasonal breeding patterns, which have been attributed to variations in sea temperature (Muthiga, 2005; Chang-Po & Kun-Hsiung, 2012). During seasons of naturally low reproductive output, farming efforts experience a decrease in production and in gamete quality. To help supplement urchin output and grow out efforts throughout the year, I suggest investigating the cryopreservation of collector urchin gametes and embryos. Cryobanking reproductive material could provide aquaculture efforts with quality reproductive material at any time of the year. Stocking cryopreserved material would also provide an insurance of sorts, in the event that normal spawning efforts should fail or that healthy urchins were otherwise unavailable. Cryopreservation of *T. gratilla* reproductive material could also serve in ecotoxicological studies and water testing. To realize this potential, a reliable protocol would need to be developed followed by further testing of the effects of cryopreservation on the urchin.

APPENDIX A

Map of research sites: Location of caging experiments around Coconut Island in Kāne‘ohe Bay. The red star corresponds to our lagoonal sites (low water motion). The orange stars represent caging sites along the reef flat (high water mixing, low predation). And the yellow stars mark caging areas along the reef slope (mixing, high predation). These three sites were used to assess post transport survival of juvenile urchins deployed in the Bay.

APPENDIX B

Growth rates of urchins size cohorts during no-choice feeding trials: Average growth rates of small, medium and large urchins while on a no-choice diet of either *A. spicifera, G. salicornia, E. denticulatum* or *K. clade B*.



REFERENCES

Alender C. B. 1964. The venom from the heads of the globiferous pedicellariae of the sea urchin *Tripneustes gratilla* (Linnaeus). Ph.D. dissertation. University of Hawai'i at Mānoa, Honolulu, HI, USA. 126 pp.

Alves F.M.A., Chícharo L.M., Serrão E., Abreu A.D. 2003. Grazing by *Diadema antillarum* (Philippi) upon algal communities of rocky substrates. *Scientia Marina*. 67: 307-311.

Anderson L.W.J. 2005. California's response to *Caulerpa taxifolia*: a model for invasive species rapid response. *Biological Invasions* 7: 1003-1016.

Anderson L. W. J. 2007. Control of invasive seaweeds. *Botanica Marina* 50: 418-437.

Arthur K. E., Balazs G. H. 2008. A Comparison of immature Green Turtle (*Chelonia mydas*) Diets among Seven Sites in the Main Hawaiian Islands. *Pacific Science* 62 (2): 205-217.

Asafu-Adjaye J., Tapsuwan S. 2008. A contingent valuation study of scuba diving benefits: Case study in Mu Ko Similan Marine National Park, Thailand. *Tourism Management* 29 (6): 1122-1130.

Ask E.I., Batibasaga A., Zertuche-Gonzalez J.A., De San M. 2003. Three decades of *Kappaphycus alvarezii* (Rhodophyta) introduction to non-endemic locations. Pp. 49-57 In: Chapman A.R.O., Anderson R.J., Vreeland V.J., Davison I.R. (eds). Proceedings of the 17th international seaweed symposium. *Oxford University Press, Oxford*.

Atalah J., Hopkins G. A., Forrest B. M. 2013a. Augmentative biocontrol in natural marine habitats: persistence, spread and non-target effects of the Sea Urchin *Evechinus chloroticus*. *PLoS ONE* 8(11): e80365. https://doi.org/10.1371/journal.pone.0080365

Atalah J., Bennette H., Hopkins G. A., Forrest B. M. 2013b. Evaluation of sea anemone *Anthothoe albocincta* as an augmentative biocontrol agent for biofouling on artificial structures. *Biofouling* 29 (5): 559-571.

Atalah J., Hopkins G. A., Fletcher L. M., Castinel A., Forrest B. M. 2015. Concepts of Biocontrol in marine environments: is there a way forward? *Management of Biological Invasion* 6 (1): 1-12.

Atalah J., Newcombe E. M., Zaiko A. 2016. Biocontrol of fouling pests: Effect of diversity, identity and density of control agents. *Marine Environmental Research* 115: 20-27.

Bahr K. D., Jokiel P. L., Toonen R. J. 2015. The unnatural history of Kāne ʻohe Bay: coral reef resilience in the face of centuries of anthropogenic impacts. *PeerJ* 3:e950 https://dx.doi.org/10.7717/peerj.950

Banner A.H. & Bailey J.H. 1970. The effects of urban pollution upon a coral reef system: a preliminary report. HIMB Technical Bulletin #25, University of Hawai'I at Mānoa. 66pp.

Batibasaga E.I., Zertuche-González J.A., de San M. 2003. Three decades of *Kappaphycus alvarezii* (Rhodophyta) introduction to non-endemic locations. *Proceedings of the International Seaweed Symposium* 17: 49-58.

Bell C. D. L., Parsons J., Austin T. J., Broderick A. C. 2005. Some of them came home: the Cayman Turtle Farm headstarting project for the green turtle *Chelonia mydas*. *Oryx* 39 (2): 137-148.

Bellwood D. R., Hughes T. P., Nystrôm M. 2004. Confronting the coral reef crisis. *Nature* 429: 827-833.

Burkepile D. E., Hay M. E. 2008. Herbivore species richness and feeding complementarity affect community structure and function on a coral reef. *PNAS* 105 (42): 16201-16206.

Caltagirone L. E. 1981. Landmark examples in classical biological control. *Annual Review in Entomology* 26: 213-232.

Cardona L., Campos P., Levy Y., Demetropoulos A., Margaritoulis D. 2010. Asynchrony between dietary and nutritional shifts during the ontogeny of green turtles (*Chelonia mydas*) in the Mediterranean. *Journal of Experimental Marine Biology and Ecology* 393 (1-2): 83-89.

Carpenter R. C. 1986. Partitioning herbivory and its effects on coral reef algal communities. *Ecological Monographs* 56 (4): 345-364.

Ceccherelli G., Piazzi L. 2005. Exploring the success of manual eradication of *Caulerpa racemosa* var. cylindracea (Caulerpales, Chlorophyta): the effect of habitat. *Cryptogamie Algologie* 26 (3): 319-328.

Chang G. C., Kareiva P. 1999. The case of indigenous generalists in biological control. pp103-115 *in* Theoretical approaches to biological control, Hawkins B. A. and Cornell H. V., editors. Cambridge University Press, New York, New York, USA.

Chang-Po C., Kung-Hsiung C. 2012. Reproductive periodicity of the sea urchin, *Tripneustes gratilla* (L.) in Taiwan compared with other regions. *International Journal of Invertebrate Reproduction* 3 (6): 309-319.

Chiappone M., Swanson D.W., Miller S.L., Smith S.G. 2002. Large-scale surveys on the Florida reef tract indicate poor recovery of the long-spined sea urchin *Diadema antillarum. Coral Reefs*. 21: 155-159.

Coles S.L., DeFelice R.C., Eldredge L.G. 2002. Nonindigenous Marine Species in Kāneʻohe Bay, Oʻahu, Hawaiʻi. *Bishop Museum Press*. 24: 1-359.

Conklin K. Y., Kurihara A., Sherwood A. R. 2009. A molecular method for identification of the morphologically plastic invasive algal genera *Eucheuma* and *Kappaphycus* (Rhodophyta, Gigartinales) in Hawaii. *Journal of Applied Phycology* 21(6): 691-699.

Conklin E. J., Smith J. E. 2005. Abundance and spread of the invasive red algae, *Kappaphycus* spp., in Kāneʻohe Bay, Hawaiʻi and an experimental assessment of management options. *Biological Invasions* 7: 1029-1039.

Coquillard P., Thibaut T., Hill D. R. C., Gueugnot J., Mazel C., Coquillard Y. 2000. Simulation of the mollusc Ascoglossa *Elysia subornata* population dynamics: application of the potential biocontrol to *Caulerpa taxifolia* growth in the Mediterranean Sea. *Ecological Modelling* 135 (1): 1-15.

Cowie R. H. 2010. Can snails ever be effective and safe biocontrol agents? *International Journal of Pest Management* 47: 23-40.

Cruz C., Segarra A. 1992. Potential for biological control of crop pests in the Caribbean. *Florida Entomologist* 75(4): 400-408.

Dafni J., Tobo R. 1987. Population structure patterns of a common red sea echinoid *Tripneustes-gratilla-elatensis*. *Israel Journal of Zoology*. 34: 3-4. 191-204.

Donahue M. J., Boland R. C., Sramek C. M., Antonelis G. A. 2001. Derilict fishing gear in the Northwestern Hawaiian Islands: Diving surveys and debris removal in 1999 confirm threat to coral reef ecosystem. *Marine Pollution Bulletin* 42 (12): 1301-1312.

Done T. J. 1992. Phase shifts in coral reef communities and their ecological impacts. *Hydrobiologia* 247: 121-131.

Doty M.S. 1961. *Acanthophora*, a possible invader of the marine flora of Hawaii. *Pacific Science*. 15: 547-552.

Doty M.S. 1977. *Eucheuma*-current marine agronomy. *The Marine Plant Biomass of the Pacific Northwest Coast*. Krauss R.W. ed, Oregon State University Press. Corvallis, OR: 203-214.

Doulman D. J. 1993. Community-based fishery management: Towards the restoration of traditional practices in the South Pacific. *Marine Policy* 17 (2): 108-117.

Dworjanyn S. A., Pirozzi I., Wenshan L. 2007. The effect of the addition of algae feeding stimulants to artificial diets for the sea urchin *Tripneustes gratilla*. *Aquaculture* 273: 624-633.

Edmonson C. H. 1946. Reef and shore fauna of Hawaii. *Bernice P. Bishop Museum Special Publication* 22: 381.

Elmqvist T., Folke C., Nyström M., Peterson G., Bengtsson J., Walker B., Norber J. 2003. Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment* 1 (9): 488-494.

Enright C., Krailo D., Staples L., Smith M., Vaughan C., Ward D., Gaul P., Borgese E. 1984. Biological control of fouling algae in oyster aquaculture. *Journal of Shellfish Research* 3:41-44.

Erwin P. M., López-Legentil S., Schuhmann P. W. 2010. The pharmaceutical value of marine biodiversity for anticancer drug discovery. *Ecological Economics* 70 (2):445-451.

Forsman Z. H., Rinkevich B., Hunter C. L. 2006. Investigating fragment size for culturing reef-building corals (*Porites lobata* and *P. compressa*) in *ex situ* nurseries. *Aquaculture* 261: 89-97.

Friedlander A., Poepoe K., Poepoe K., Helm K., Bartram P., Maragos J., Abbott I. 2002. Application of Hawaiian traditions to community-based fishery management. *Proceedings of the 9th International Coral Reef Symposium, Bali, Indonesia* 2: 813-818.

Gibo C., Letsom T., Westbrook C. 2012. Effects of temperature, salinity, pH, reef size, and *Tripneustes gratilla* on the distribution of *Montipora dilatata* in Kāneʻohe Bay. Final class report to NOAA species of Concern, Honolulu. http://www.fpir.noaa.gov/Library/PRD/SOC/M.%20dilatata%20SOC%20Final%20Report%207-2012.pdf

Glasby T. M., Gibson P. T., Kay S. 2005a. Tolerance of Marine Alga *Caulerpa taxifolia* to burial by sediment. *Aquatic Botany* 82: 71-81.

Glasby T. M., Greese R. G., Gibson P. T. 2005b. Experimental use of salt to control the invasive marine alga *Caulerpa taxifolia* in New South Wales, Australia. *Biological Conservation* 122 (4): 573-580.

Glenn E.P., Doty M.S. 1990. Growth of the seaweeds *Kappaphycus alvarezii, K. striatum* and *Eucheuma denticulatum* as affected by the environment in Hawai'i. *Aquaculture.* 84: 245-255.

Goddard J. H. R., Torchin M. E., Kuris A. M., Lafferty K. D. 2005. Host specificity of *Sacculina carcini*, a potential biological control agent of the introduced European green crab *Carcinus maenas* in California. *Biological Invasions* 7 (6): 895-912.

Godwin S., Rodgers K. S., Jokiel P. L. 2006. Reducing Potential Impacts of Invasive Marine Species in the Northwestern Hawaiian Islands Marine National Monument. Report to: *Northwestern Hawaiian Islands Marine National Monument Administration*, 1-66

Gumbo R. J., Ross G., Cloete E. T. 2008. Biological control of *Microcystis* dominated harmful algal blooms. *African Journal of Biotechnology* 7 (25): 4765-4773.

Hawkins J. P., Roberts C. M. 2004. Effects of Artisanal Fishing on Caribbean Coral Reefs. *Conservation Biology* 18 (1): 215-226.

Hédouin L., Metian M., Gates R. 2011. Ecotoxicological approach for assessing the contamination of a Hawaiian coral reef ecosystem (Honolua Bay, Maui) by metals and metalloids. *Marine Environmental Research* 71: 149-161.

Hill M. P. 1998. Life history and laboratory host range of *Stenopelmus rufinasus*, a natural enemy of *Azolla filiculoides* in South Africa. *Biocontrol* 43 (2): 215-224.

Hoddle M. S. 2004. Restoring balance: Using exotic species to control invasive exotic species. *Conservation Biology* 18: 38-49.

Hoegh-Guldberg O., Mumby P. J., Hooten A. J., Steneck R. S., Greenfield P., Gomez E., Harvell C. D., Sale P. F., Edwards A. J., Caldeira K., Knowlton N., Eakin C. M., Iglesias-Prieto R., Muthiga M., Bradbury R. H., Dubi A., Hatziolos M. E. 2007. Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science* 318 (5857): 1737-1742.

Hofkin B. V., Koech D. K., Oumaj J., Loker E. S. 1991. The North American crayfish *Procambarus clarkii* and the biological control of schistosome-transmitting snails in Kenya: Laboratory and field investigations. *Biological Control* 1 (3): 183-187.

Holland B.S., Taylor C., Sugiura S. 2012. Tracking behavior in the snail *Euglandina rosea*: First evidence of preference for endemic vs. biocontrol target pest species in Hawaii. *American Malacological Bulletin* 30 (1): 153-157.

Hoover J. P. (2002) Hawai'i's Sea Creatures: A Guide to Hawai'i's Marine Invertebrates. Mutual Publication, Honolulu, HI.

Howarth F. G. 1983. Classical Biocontrol: Panacea or Pandora's Box. *Proceedings, Hawaiian Entomological Society* 24: 239-244.

Howarth F. G. 1985. Impact of alien land arthropods and mollusks on native plants and animals in Hawai'i. *Hawaii's Terrestrial Ecosystems: Preservation and Management.* Stone C.P. and Scott J.M. ed, University of Hawaii's Cooperative National Park Resources Study Unit. . Honolulu, HI: 149-178.

Howarth F. G. 1991. Environmental impacts of classical biological control. *Annual Review of Entomology* 36: 485-509.

Hughes T. P. 1994. Catastrophes, Phase Shifts, and Large-Scale Degradation on a Caribbean Coral Reef. *Science* 265 (5178): 1547-1551.

Hunter C. L., Evans C. W. 1995. Coral reefs in Kaneohe Bay, Hawaii: two centuries of western influence and two decades of data. *Bulletin of Marine Science 57*(2): 501-515.

Jackson J. B. C., Kirby M. X., Berger W. H., Bjorndal K. A., Botsford L. W., Bourque B. J., Bradbury R. H., Cooke R., Erlandson J., Estes J. A., Hughes T. P., Kidwell S., Lange C. B., Lenihan H. S., Pandolfi J. M., Peterson C. H., Steneck R. S., Tegner M. J., Warner R. R. 2001. Historical Overfishing and Recent Collapse of Coastal Ecosystems. *Science* 293 (5530): 629-638.

Jellyman P. G., Clearwater S. J., Clayton J. S., Kilroy C., Blair N., Hickey C. W., Biggs B. J. F. 2011. Controlling the invasive diatom *Didymosphenia geminata*: An exotic assessment of four potential biocides. *Archive of Environmental Contamination and Toxicology* 61: 115-127.

Kay E. A. 1994. Marine ecosystems in the Hawaiian Islands. *A Natural History of the Hawaiian Islands: Selected Reading II,* EA Kay (ed). University of Hawai 'i Press: USA, 187-195.

Kamalakannan B., Jeevamani J. J. J., Nagendran N. A., Pandiaraja D., Chandrasekaran S. 2014. Impact of removal of invasive species *Kappaphycus alvarezii* from control reef ecosystem in Gulf of Mannar, India. *Current Science* 106 (10): 1401-1408.

Kimbro D. L., Cheng B. S., Grosholz E. D. 2013. Biotic resistance in marine environments. *Ecology Letters* 16: 821-833.

Kohn A.J. 1961. Studies on Spawning Behavior, Egg Masses, and Larval Development in the Gastropod Genus *Conus*, Part I Observations on Nine Species in Hawai'i. *Pacific Science*. 15: 163-179.

Kuffner I. B., Walters L. J., Becerro M. A., Paul V. J., Ritson-Williams R., Beach K. S. 2006. Inhibition of coral recruitment by macroalgae and cyanobacteria. *Marine Ecology Progress Series* 323: 107-117.

Kuster C., Vuki V. C., Zann L. P. 2005. Long-term trends in subsistence fishing patterns and coral reef fisheries yield from a remote Fijian island. Fisheries Research 76 (2): 221-228.

Lafferty K. D., Kuris A. M. 1996. Biological control of marine pests. *Ecology* 77 (7): 1989-2000.

Lam A. K. Y., Prepas E. E., Spink D., Hrudey S. E. 1995. Chemical control of hepatoxic phytoplankton blooms: Implications for human health. *Water Research* 29 (8): 1845-1854.

Lawrence J.M., Agatsuma Y. 2001. The Ecology of *Tripneustes gratilla*. In: Lawrence J.M. (ed) *Edible Sea Urchins: Biology and Ecology*. Elsevier, Amsterdam. p 514.

Leal M. C., Calado R., Sheridan C., Alimonti A., Osinga R. 2013. Coral aquaculture to support drug discovery. *Trends in Biotechnology* 31 (10): 555-561.

Lessios H. A. 1988. Mass Mortality of *Diadema Antillarum* in the Caribbean: What Have We Learned? *Annual Review of Ecology and Systematics* 19: 371-393.

Lessios H. A. 2016. The Great *Diadema antillarum* Die-Off: 30 Years Later. *The Annual Review of Marine Science* 8: 1.1-1.17.

Lessios H. A., Kane J., Robertson D. R. 2003. Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. *Evolution* 57: 2026-2036.

Lodeiros C., García N. 2004. The use of sea urchins to control fouling during suspended culture of bivalves. *Aquaculture* 231 (1-4): 293-298.

Louda S. M., Stiling P. 2004. The Double-Edged Sword of Biological Control in Conservation and Restoration. *Conservation Biology* 18: 50-53.

Lyons D. A., Scheibling R. E. 2009. Range expansion by invasive marine algae: rates and patterns of spread at a regional scale. *Diversity and Distribution* 15 (5): 762-775.

Malavé C., Freites L., Lodeiros C., Mendoza J., Troccoli L., Dale A. W. 2012. Annual recruitment, predation rates and biocontrol of *Linatella caudata* (Mollusca: Gastropoda) in suspended enclosure culture of the pearl oyster *Pinctada imbricada*. *Aquaculture* 354-355: 75-83.

Marks L. M., Reed D. C., Obaza A. K. 2017. Assessement of control methods for invasive seaweed *Sargassum horneri* in California, USA. *Management of Biological Invasion* 8 (2): 205-2013.

McAllister D. E. 1991. What is the status of coral reef fishes? *Sea Wind* 5: 14-18.

McClanahan T. R., Hendrick V., Rodrigues M. J., Polunin N. V. C. 1999. Varying response of herbivorous and invertebrate-feeding fishes to macroalgal reduction on a coral reef. *Coral Reefs* 18: 195-203.

McConnachie A. J., de Wit M. P., Hill M. P., Byrne M. J. 2003. Economic evaluation of the successful biological control of *Azolla filiculoides* in South African. *Biocontrol* 28: 25-32.

McCook L., Jompa J., Diaz-Pulido G. 2001. Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs* 19 (4): 400-417.

McDermid K.J., Stuercke B., Haleakala O.J. 2005. Total dietary fiber content in Hawaiian marine algae. *Botanica Marina*. 48: 437-440.

McDermid K.J., Stuercke B., Balazs G.H. 2007. Nutritional composition of marine plants in the diet of the green sea turtle (*Chelonian mydas*) in the Hawaiian Islands. *Bulletin of Marine Science*. 81: 55-71.

McManus J. W., Meñez L. A. B., Kesner-Reyes K. N., Vergara S. G., Ablan M. C. 2000. Coral reef fishing and coral-algal phase shifts: implications for global reef status. *ICES Journal of Marine Science* 57 (3): 572-578.

Michaud J. P. 2002. Classical Biological Control: A Critical Review of Recent Programs Against Citrus Pests in Florida. *Entomological Society of America* 94(5): 531-540.

Miller A. W., Chang A. L., Cosentino-Manning N., Ruiz G. M. 2004. A new record and eradication of the Northern Atlantic alga *Ascophyllum nodosum* (Phaeophyceae) from San Francisco Bay, California, USA. *Journal of Phycology* 40 (6): 1028-1031.

Moberg F., Folke C. 1999. Ecological goods and services of coral reef ecosystems. *Ecological Economics* 29 (2): 215-233.

Mumby P. J., Harborne A. R., Brumbaugh D. R. 2011. Grouper as a Natural Biocontrol of Invasive Lionfish. PLoS ONE 6 (6): e21510. https://doi.org/10.1371/journal.pone.0021510

Mumby P.J., Hedley J.D., Zychaluk K., Harborne A.R., Blackwell P.G. 2006. Revisiting the catastrophic die-off of the urchin *Diadema antillarum* on Caribbean coral Reefs: Fresh insights on resilience from a simulation model. *Ecological Modeling*. 196: 131-148.

Munro J. L. (ed.). 1983. Caribbean Coral Reef Fishery Resource (ICLARM Stud. Rev. 7). *ICLARM*, Manila, Philippines, 276 pp.

Murphy J. W. A., Richmond R. H. 2016. Changes to coral health and metabolic activity under oxygen deprivation. PeerJ 4:e1956 https://doi.org/10.7717/peerj.1956

Muthiga N. A. 2005. Testing for the effects of seasonal and lunar periodicity on the reproduction of the edible sea urchin *Tripneustes gratilla* (L) in Kenyan coral reef lagoons. *Hydrobiologia* 549: 57-64.

Ogden N. B., Ogden J. C., Abbott I. A. 1989. Distribution, abundance and food of sea urchins on a leeward Hawaiian reef. *Bulletin of Marine Science* 45(2): 539-549.

Parker J. D., Burkepile D. E., Hay M. E. 2006. Opposing effects of native and exotic herbivores on plant invasions. *Science* 301: 1459-1561.

Parry G. D. 2017. Potential for biocontrol of the exotic starfish, *Asterias amurensis*, using a native starfish. *Biological Invasions* 19 (7): 2185-2196.

Peller J. R., Whitman R. L., Griffith S., Harris P., Peller C., Scallzitti J. 2007. TiO_2 as a photocatalyst for control of the aquatic invasive alga, *Cladophora*, under natural and artificial light. *Journal of Photochemistry and Photobiology A: Chemistry* 186 (2-3): 212-217.

Peters K.J., Amsler C.D., Amsler M.O., McClintock J.B., Iken K.B., Baker W.J. 2002. The Effects of Thallus Toughness and Nutritive Quality on Palatability of Antarctic Macroalgae. *Journal of Phycology*. 38: 29-30.

Reguero B. G., Beck N. W., Agostini V. N., Kramer P., Hancock B. 2018. Coral reefs for coastal protection: A new methodological approach and engineering case study in Grenada. *Journal of Environmental Management* 210: 146-161.

Rodgers S.K., Cox E.F. 1999. Rate of Spread of Introduced Rhodophytes *Kappaphycus alvarezii*, *Kapphaphycus striatum*, and *Gracilaria salicornia* and Their Current Distribution in Kāneʻohe Bay, Oʻahu, Hawaiʻi. *Pacific Science*. 53: 232-241.

Ross K. A., Thorpe J. P., Brand A. R.2004. Biological control of fouling in suspended scallop cultivation. *Aquaculture* 229 (1-4): 99-116.

Russel D.J. 1983. Ecology of the Imported Red Seaweed *Eucheuma striatum* Schmitz on Coconut Island, Oʻahu, Hawaiʻi. *Pacific Science*. 37: 87-107.

Russel D.J., Balazs G.H. 1994. Colonization by the alien marine alga *Hypnea musciformis* (Wulfen) J.Ag.(Rhodophyta: Gigartinales) in the Hawaiian islands and its utilization by the green turtle, *Chelonia mydas* L. *Aquatic Botany* 47 (1): 53-60

Russel D.J., Balazs G.H. 2009. Dietary Shifts by Green Turtles (*Chelonia mydas*) in the Kāneʻohe Bay Region of the Hawaiian Islands: A 28-Year Study. *Pacific Science*. 63: 181-192.

Sandin S.A., Walsh S.M., Jackson J.B.C. 2010. Prey release, trophic cascades, and phase shifts in tropical nearshore ecosystems. Pp. 71 – 90 In Terborph J., Estes J.A. (eds.) *Trophic Cascades: Predators, Prey, and the Changing Dynamics of Nature. Island Press*, Washington D.C.

Santelices B. 2004. A comparison of ecological responses among aclonal (unitary), clonal and coalescing macroalgae. *Journal of Experimental Marine Biology and Ecology* 300: 31-64.

Selkoe K. A., Halpern B. S., Toonen R. J. 2008. Evaluating anthropogenic threats to the Northwestern Hawaiian Islands. *Aquatic Conservation: Marine and Freshwater Ecosystems* 18: 1149-1165.

Seymour S., Paul N.A., Dworjanyn S.A., de Nys R. 2013. Feeding preference and performance in the tropical sea urchin *Tripneustes gratilla. Aquaculture*. 400-401: 6-13.

Sheppard C. R. C., Ateweberhan M., Bowen B. W., Carr P., Chen C. A., Clubbe C., Craig M. T., Ebinghaus R., Elbe J., Fitzsimmons N., Gaither M. R., Gan C-H., Gollock M., Guzman N., Graham N. A. J., Harris A., Jones R., Keshavmurthy S., Koldewey H., Lundin C. G.,

Mortimer J. A., Obura D., Pfeiffer M., Price A. R. G., Purkis S., Raines P., Readman J. W., Riegl B., Rogers A., Schleyer M., Seaward M. R. D., Sheppard A. L. S., Tamelander J., Turner J. R., Visram S., Vogler C., Vogt S., Wolschke H., Yang J. M. C., Yang S-Y., Yesson C. 2012. Reefs and islands of Chagos Archipelago, Indian Ocean: why it is the world's largest no-take marine protected area. Aquatic Conservation: Marine and Freshwater Ecosystems 22 (2): 232-261.

Sherwood A. R., Kurihara A., Conklin K. Y., Sauvage T., Presting G. G. 2010. The Hawaiian Rhodophyta Biodiversity Survey (2006-2010): a summary of principal findings. *BMC plant biology* 10(1): 258.

Sigee D. C., Glenn R., Andrews M. J., Bellinger E. G., Butler R. D., Epton H. A. S., Hendry R. D. 1999. Biological control of cyanobacteria: principles and possibilities. I*n* The Ecological Basis for Lake and Reservoir Management, Harper D. M., Brierly, Ferguson A. J. D., Philips G. (eds.), Hydrobiologia 395(396): 161-171.

Simberloff D., Stiling P. 1996. How risky is biological control? *Ecology* 77 (7): 1965-1974.

Simberloff D., Dayan T., Jones C., Ogura G. 2000. Character Displacement and Release in the Small Indian Mongoose, *Herpestes javanicus*. *Ecology* 81 (8): 2086-2099.

Smith C. M., Walters L. J. 2002. Fragmentation as a Strategy for *Caulerpa* Species: Fate of Fragments and Implications for Management of an Invasive Weed. *Marine Ecology* 20 (4): 307-319.

Smith J. E., Hunter C. L., Smith C. M. 2002. Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Islands. *Pacific Science 56*(3): 299-315.

Smith J.E., Hunter C.L., Conklin E.J., Most R., Sauvage T., Squair C., Smith C.M. 2004. Ecology of the Invasive Red Alga *Gracilaria salicornia* (Rhodophyta) on Oʻahu, Hawaiʻi. *Pacific Science* 58: 325-343.

Spalding M. D., Grenfell A. M. 1997. New estimates of global and regional coral reef areas. *Coral Reefs* 16(4): 225-230.

Sterner R.W., Hessen D.O. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual review of Ecology and Systematics* 1-29.

Stiling P., Cornelissen T. 2005. What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological Control* 34: 236-246.

Stimson J., Larned S. T., Conklin E. 2001. Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga *Dictyophaeria caevernosa* in Kāneʻohe Bay, Hawaiʻi. *Coral Reefs* 19: 343-357.

Stimson J., Cunha T., Philippoff J. 2007. Food preference and related behavior of the browsing sea urchin *Tripneustes gratilla* (Linnaeus) and its potential for use as a biological control agent. *Marine Biology* 151: 1761-1772.

Sumi C. B. T., Scheibling R. E. 2005. Role of grazing by sea urchin Strongylocentrotus droebachiensis in regulating invasive algal *Codium fragile* ssp. *tomentosoides* in Nova Scotia. *Marine Ecology Progress Series* 292: 203-212.

Sun B., Aye N. N., Wang X. M., Zhu X. M., Sato M. 2011. Eradication of invasive organisms from ballast water with electrodeless pulsed-discharge hybrid reactor. *IEEE Transaction of Industry Applications* 47 (3): 1079-1085.

Symondson W. O. C., Sunderland K. D., Greenstone M. H. 2002. Can Generalist Predators be Effective Biocontrol Agents? *Annual Review in Entomology* 47: 561-594.

Taylor R.B., Sotka E., Hay M.E. 2002. Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing. *Oecologia* 132 (1): 68-76.

Theil M., Westphalen G., Collings G., Cheshire A. 2007. *Caulerpa taxifolia* responses to hyposalinity stress. *Aquatic Botany* 87 (3): 221-228.

Thibaut, T., Meinesz A. 2000. Are the Mediterranean ascolglossan molluscs *Oxynoe olivacea* and *Lobiger serradifalci* suitable agents for biological control against the invading tropical alga *Caulerpa taxifolia*? *Comptes Rendues de l'Académie des Sciences* 323 (5): 477-488.

Thomas F. 1994. Physical properties of gametes in three sea urchin species.*The Journal of Experimental Biology 194*(1): 263-284.

Thornber C., Stachowicz J.J., Gaines S. 2006. Tissue type matters: selective herbivory on different life history stages of an isomorphic alga. *Ecology* 87 (9): 2255-2263.

Thresher R. E., Kuris A. M. 2004. Options for managing invasive marine species. *Biological Invasions* 6: 295-300.

Vaïtilington D., Rasolofonirina R., Jangoux M. 2007. Feeding preferences, seasonal gut repletion indices, and diel feeding patterns of the sea urchin *Tripneustes gratilla*

(Echinodermata: Echinoidea) on a coastal habitat off Toliara (Madagascar). *Marine Biology* 143: 451-458.

Valentine J. P., Edgar G. J. 2010. Impacts of a population outbreak of the urchin *Tripneustes gratilla* amongst Lord Howe Island coral communities. *Coral Reefs* 29: 399-410.

Van Heukelem L. 2016. Does the initial diet of hatchery-reared *Tripneustes gratilla* (Linnaeus) impact their effectiveness as biocontrol for invasive seaweeds. *Unpublished Master's Thesis*. University of Hawai'i at Mānoa, Honolulu, Hawai'i.

Wagner D., Kahng S. E., Toonen R. J. 2009. Observations of the life history and feeding ecology of a specialized nudibranch predator (*Phyllodesmium poindimiei*), with implications for biocontrol of an invasive octocoral (*Corijoa riisei*) in Hawaii. *Journal of Experimental Marine Biology and Ecology* 372(1-2): 64-74.

Watson D.C., Norton T.A. 1985. The physical characteristics of seaweed thalli as deterrents to littorine grazers. *Botanica Marina* 28 (9): 383-388.

Weijerman M., Most R., Wong K., Beavers S. 2008. Attempt to control the invasive red alga *Acanthophora spicifera* (Rhodophyta: Ceramiales) in a Hawaiian fishpond: An assessment of removal techniques and management options. *Pacific Science* 62 (4): 517-532.

Weis J.S., Butler C.A. 2009. Salt Marshes: a natural and unnatural history. *Rutgers University Press*: 159-160.

Wilkinson C. R. 1999. Global and local threats to coral reefs functioning and existence: review and predictions. *Marine and Freshwater Research* 50 (8): 867-878.

Williams S. L., Smith J. E. 2007. A Global Review of the Distribution, Taxonomy, and impact of introduced Seaweeds. *Annual Review of Ecology, Evolution and Systematics* 38: 327-359.

Wotton D. M., O'Brien C., Stuart M. D., Fergus D. J. 2004. Eradication success down under: heat treatment of a sunken trawler to kill the invasive seaweed *Undaria pinnatifida*. *Marine Pollution Bulletin* 49 (9-10): 844-849.

Wu D. H., You H., Du J. X., Chen C. A., Jin D. R. 2011. Effects of UV/Ag-TiTO2/O-3 advanced oxidation on unicellular green alga *Dunaliella salina*: Implications for removal of invasive species from ballast water. *Journal of Environmental Science* 23 (3): 513-519.

Wylie C.R., Paul V.J. 1988. Feeding preferences of the surgeonfish *Zebrasoma flavescens* in relation to chemical defenses of tropical algae. *Marine Ecology Progress Series* 45: 23-32.